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CHROMOSOME STRUCTURE[1]
ON COILING IN CHROMOSOMES[2]

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THE present paper is an attempt to construct a mechanical model of the coiling of chromonemata. As a speculation standing between the observational facts obtained with the aid of the microscope and the existing knowledge of biochemistry of chromosomes the present image is to serve the biochemist as well as the microscopist as a reference point for further approaches.

There exist with regard to the mechanism of coiling two main schools of speculation which may be termed the matrical and the molecular. Exponents of the former are Huskins, Kuwada, Sax and others; of the latter, Darlington.

Wilson and Huskins (1939) write:

> As the simplest possibility, let us consider the space-limiting factor to be a pellicle[3] which remains nearly stationary in size while the chromonema is elongating. Let us further assume, in accord with morphological evidence (apart from functional considerations), that this pellicle forms prior to the beginning of spiralization and in some manner disappears prior to the unravelling of the spiral in first pollen-grain division prophase.

[1] Presented at the joint symposium of the American Society of Zoologists and the Genetics Society of America in conjunction with the American Association for the Advancement of Science at Richmond, Virginia, December 28, 1938.

[2] Approved by the Director of the N. Y. State Agricultural Experiment Station as Journal Paper 307. Jan. 6, 1939.

[3] Darlington, who, contrary to us, doubts the existence of a "matrix," agrees (1936b) that: "There are several grounds—chiefly non-morphological—for assuming that the chromosome thread has some sort of pellicle."

Some of the expected properties of the spiral which this method of formation would produce are:

(1) If elongation is gradual and uniform throughout the length of the chromonema and coiling is not caused by an internal torsion, an irregular zigzag would be expected in the early stages of spiralization.

(2) Any point of interruption of the spiral such as chiasmata, the attachment region, bends in the pellicle, or any lack of homogeneity in the chromonema and its matrix, might cause changes in the direction of coiling (with random frequency).

(3) Chromatids, closely associated during spiralization, should coil in the same direction.

(4) Uncoiling would be expected to be gradual.

Kuwada (1937) writes:

The mechanism of chromonema coiling we assume to occur in two steps. (1) The chromonema threads are brought to a twisted state by intensification of the internal tendency by anisotropic swelling. (2) When imbibition water is distributed evenly the threads are forced to untwist. The untwisting is realized by transformation of the twist into a spiral, the matrix playing a great rôle in this transformation and also bringing the spiral into the regular form. When the contracting force of the matrix is lost spirals become loose. The internal twisting (1) and the matrical force (2) which contracts are regarded as two inseparable factors.

Kuwada also introduces the plant *tendril* to illustrate reversals of direction of coiling which compensate for twists in one direction by corresponding ones in the other.

Sax and Humphrey (1935) state:

. . . coiling can be simulated by compressing two closely associated flexible wires in a glass tube while the ends of the wires are not permitted to rotate. The chromatids are undoubtedly somewhat elastic and flexible as shown by their behavior at division. They may be prevented from rotating within the pellicle by the association between the fiber attachment points and the pellicle. Inhibition of rotation at the distal ends of the chromosomes may also be affected to some extent by the terminal or subterminal chiasmata.

(This discussion pertains to meiosis primarily.) Thus the chromonema has elastic properties and is passively helicated. The matrix is responsible for contraction and spiralling.

In the scheme of Kuwada and in the now abandoned scheme of Huskins and Smith anisotropic growth is responsible for coiling. Sax does not suggest what determines the pitch and the regularity of the spiral.

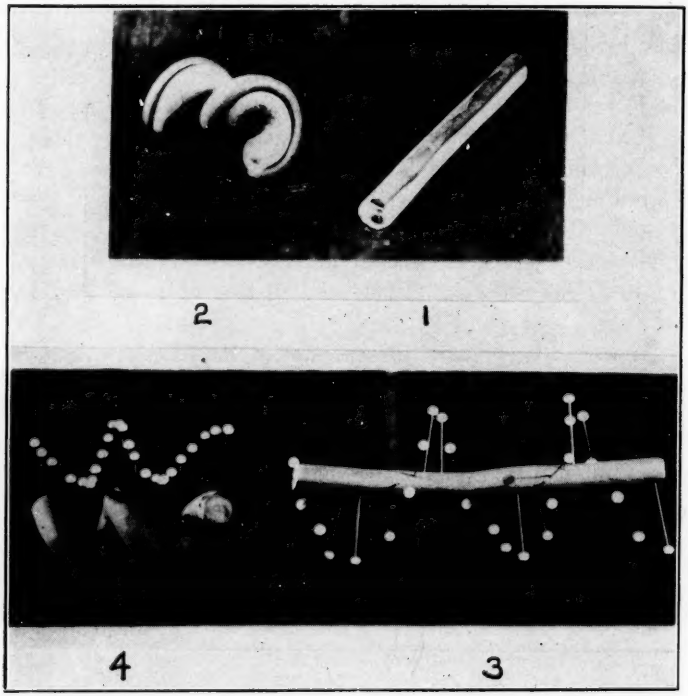
In contrast to all the foregoing Darlington (1935) links the reason for coiling to some basic asymmetry of the

molecules constituting the gene thread. This is implied in his term *molecular coil.* He states that the spiral (coiling of chromatids or helication) arises by an internal twisting of the thread in opposite direction to the spiral assumed, and that since the molecular spiral must determine both major and minor spirals, these must be in the same direction and opposite to the twist that conditions or determines them. One may thus contrast the "matrical" and the "molecular" schemes of coiling, and the essential features of these schemes are illustrated in two pairs of models, Figs. 1, 2 and 3 and 4.

It would be difficult to construct a model of the entire chromosome including matrix, chromonema and gene string. Hence the matrical space has been omitted in the models of Figs. 1 to 4 but may be imagined by loosely enclosing, in a mental picture, each model in a Cellophane bag. In Fig. 1 is shown a cylindrical body, which stands for the chromonema proper. Its axis constitutes the genonema. The model carries a cleat running along its entire length; this stands for a heterogenic sector of the chromonema and is attached to the outside of the cylinder to make it more obvious. Chromonemata in the microscope being cylindrical, such a sector if existing would not project from the periphery of the chromonema. Fig. 2 shows the same model as Fig. 1, but the chromonema is now helicated. This process may have come about by expansion of the cleat, which would at first tend to spiralize the thread, but being confined in the matrical bag a helix will result.

In this model the cleat may be omitted. If the matrix is rigid and the chromonema elongates within it and if the latter is thought of as having physical properties of a comparable rubber rod, a coil will result. This is the picture of Wilson and Huskins (1939). Unfortunately not all investigators agree that the thread is straight and relatively short during prophase and elongates toward metaphase.

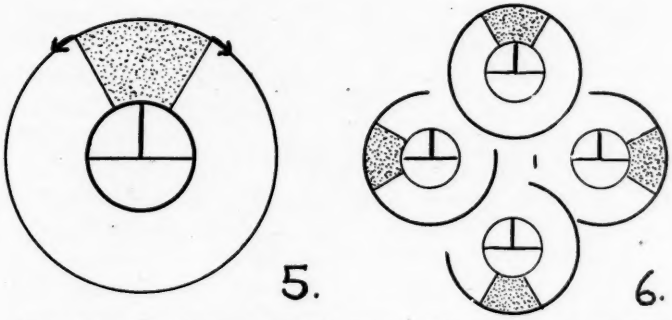
In such a structure as discussed above the coiling of the straight thread into a helix would result in a twist of the thread. If, on the other hand, one wishes to maintain ulti-



Figs. 1 and 2. Models of matrical concept. Clay models of coiling as occasioned by sectorial (heterogonic) swelling. Fig. 1 is a straight thread (cylinder) with heterogonic sector (cleat) which upon swelling will cause coiling (Fig. 2) if the structure is confined in a limited cylindrical space.

Figs. 3 and 4. Models of the nemameric concept. Clay models of nemameric (molecular) coiling. In Fig. 3 pins are arranged in a helix around a central thread. The pins represent points of contact for external forces with individual thread units (nemameres). Fig. 4 shows how an external force oriented in space (polarized) will pull successive pinheads in one general direction and will cause the thread to coil in the opposite direction of the nemameric twist. The action of the polarizing force in the model is assumed to have started at one end.

mate units of the straight thread in the identical orientation in space in spite of coiling, the heterogonic sector (the cleat of the model in Figs. 1 and 2) will have to rotate around the core. This is illustrated in Figs. 5 and 6, which are diagrammatic cross-sections of a chromonema of the



Figs. 5 and 6. Models of matrical concept. Diagrammatic cross-sections through a genonema as described in Figs. 1 and 2. Fig. 5 is the cross-section of the straight thread. Fig. 6 shows 4 successive cross-sections of one gyre of the helix in which the heterogonic sector must migrate around the center if the latter remains untwisted in space. Heterogonic sector dotted. Arrows indicate alternative directions of migration.

type illustrated in the models of Figs. 1 and 2. The cleat or heterogonic sector is shown as a dotted area and the arrows in Fig. 5 indicate that this sector may migrate around the center in either one of two directions. Fig. 6 gives four consecutive cross-sections of the chromonema, taken at distances one quarter of a turn of the helix apart from one another. It may be seen that the shaded area is always on the outside of the central figure. One may ask why it is necessary to assume that the coiled gene string maintain its orthotropic (untwisted) orientation in space: If coiled threads can multiply in the coiled condition and separate freely without uncoiling, *cf.* ring-chromosomes, multiplication and hence orientation would have to be orthotropic for all units of a given thread. According to Huskins (1935) and according to Kuwada (1937), the gene string is twisted in the relaxed state, in the coiled state it is untwisted. Speaking teleologically the chromonemata coil so that daughter chromonemata may separate without entanglement. It is thus tacitly assumed or implied by Huskins and by Kuwada that the gene strings at the time of multiplication (interphase) may be wrapped around one another. In Kuwada's scheme it is not stated clearly what determines the twisted sector in the relaxed thread.

Darlington (1935) introduced the molecular spiral. One assumes that consecutive molecules of the gene string show some systemic asymmetry which will make the thread "twisted." This principle and the superstructure of speculation suggested by Darlington have been generally rejected primarily because he fails to suggest a mechanical principle which will explain randomness of direction of coiling and random reversals of direction of coiling.

In the following I intend to resurrect the principle of the molecular coil but to endow it with properties which will make the direction of coiling facultatively determined anew at each period of gene multiplication. Certain changes in terminology suggest themselves which evolve from the existing uncertainty about orders of magnitude and mechanical qualities of molecular aggregates.

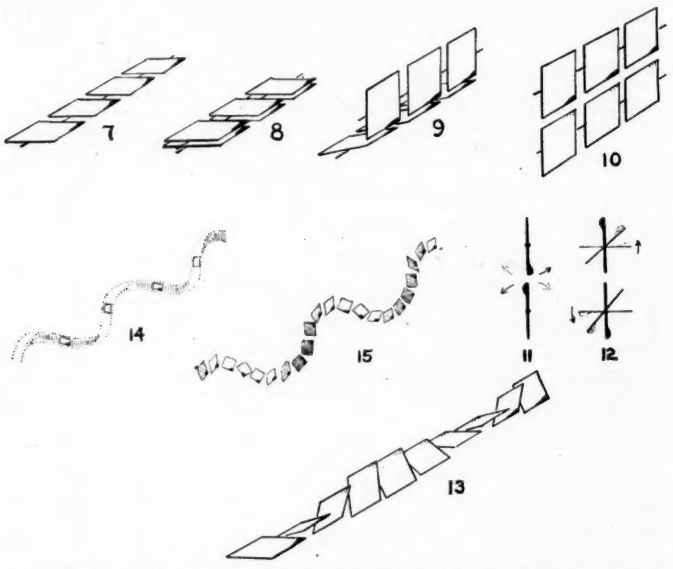
"Molecular spiral" implies that the basic units of the gene string are single molecules and these are asymmetrically built. I would like to think of the genonema as being built up of units which may be aggregates and call the basic unit of the gene string a *nemamere*. The nemamere which may be a fibril would stand in size between the gene and the chromomere, the latter being a visible unit, while the former, in ordinary light, is not.

Figs. 3 and 4 illustrate the principle of the nemameric helix (molecular spiral) which coils the thread by untwisting. The clay bar is the main axis of the thread which is made up of individual units; in this model each unit consists of a pin and a piece of the clay bar. The individual units are joined semi-rigidly. The orientation shown in Fig. 3 is called the nemameric twist since each unit is twisted in space relative to the next one. A helix is formed by the axial thread if the pins are forced into a parallel orientation. Thus a coil "results" from the twist. If the nemameric twist is sinistrorse the resultant coil will be dextrorse and *vice versa*. In the model the series of pinheads stand for contact points on which directional forces may act. This model represents my interpretation of Darlington's (1935) description.

In the "relaxed state" (Darlington) the chromonema of this model is straight in space, but, as shown in the model, twisted with regard to the pinheads projecting from it. According to my own interpretation a thread in this condition is not completely relaxed. Further relaxation might undo also the spiral of pinheads by separating individual unit parts enough so as to overcome the effect of their individual geometric asymmetry. Any orthotropic attractive force starting from a given point and acting on the pinheads will tend to throw the thread into a "reverse" spiral. As is seen from Fig. 4 the pinheads thus come much closer together in space. If there is any repulsion between them and yet oriented attraction from the outside the thread will lengthen while it is coiling. This actually occurs in Trillium, according to Wilson and Huskins (1939). It may be noted from Fig. 4 that the pinheads form an orderly helix which is detached and completely separated in space from the original thread around which the pinheads previously (Fig. 3) formed a helical track. Hence daughter threads, if wrapped around one another when first formed, would become free of each other through their combined helication. What might be expected of the unit parts of a genonema of the general type illustrated in Figs. 3 and 4 and how can one obtain random coiling in a model of this kind?

This is my modification of Darlington's principle:

In the diagrams 7 to 13, nemameres are pictured as flat rectangles with a projection called x-point in one corner. This x-point will, when nemameres are aligned closely (primary contraction), allow successive units rotational end-to-end freedom around the main axis of the genonema only through, *e.g.*, 320 degrees of the arc. In the completely relaxed state nemameres are so far apart that they form a flat band (Fig. 7). Nemameres multiply by reproducing themselves in space according to Figs. 8, 9 and 10. They are pictured as unfolding as the leaves of a book. The x-points cause the respective edges which carry them to break apart last. At this time there comes into play



Figs. 7–15. Models of the nemameric concept. Fig. 7 shows a piece of a genonema made up of flat nemameres carrying in one corner an x-region which serves as a point of contact with external forces. The genonema is now extended or relaxed and not coiled. Each nemamere has a leading (away from the observer) and a trailing edge, an x-point edge and a neutral edge. In Fig. 8 each nemamere has multiplied, forming an identical replica of itself in space below or above itself. In Fig. 9, daughter genonemata separate by unfolding, the x-point edges of the nemameres acting as hinges on a door or as the back of a book on two leaves. Fig. 10 shows daughter nemameres ready to separate but still held in position by attraction of x-point edges. Nemameres with backs toward the observer are shown with dotted x-points. In Figs. 11 and 12, leading and trailing edges of nemameres are shown in projection. The thickening indicates the position of the x-points. As genonemata separate mutual attraction of sister x-points gives way to mutual repulsion, which causes sister nemameres to rotate away from one another in one of two opposite directions. Solid arrows signify sinistrorse, dotted arrows dextrorse mutual repulsion. The reaction spreads from one unit to the next, piling successive nemameres "upon" one another, as indicated in Fig. 12 for three successive nemameres. Leading edges, due to their x-points will now repel the next trailing edge enough to prevent it from coming to perfect alignment. Fig. 13. As a result of the reaction shown in Figs. 11 and 12 daughter genonemata will show an isodirectional nemameric twist. Pairs of sister genonemata will be twisted dextrorsely or sinistrorsely. The twisted genonema shown in Fig. 13 is a result of shortening of the axis of the genonema or an expansion of the nemameres. The thread is now no

rotational repulsion between daughter x-point edges, and this will result in these edges revolving away from one another in one of two directions. Either direction may be chosen at random by the first pair of nemameres separating from one another. The projection of this movement is shown in diagram 11, in which the line stands for the leading and trailing edge of the nemamere the thickening for the x-point. Such a reaction is believed to start from certain points and spread from there to adjacent nemameres as the reaction in a chain might spread from one link to the next. Diagram 12 shows the result of such an event. At the same time when daughter nemameres separate, primary contraction within threads may occur. Successive nemameres will be stacked upon one another if the x-points now function to give each thread the final structure shown in Fig. 13, in which we now recognize the molecular spiral or in present terminology the nemameric twist. The nemameric twist of Fig. 13 may be transformed into a coil by expansion of the nemameres and intussusception or heterogonic growth of the x-point edges or by further (secondary) contraction. Thus a coil would be formed in which the gene string would be twisted (Fig. 14). There is, however, another alternative.

By exertion of an external polarizing force acting to

longer relaxed; in Fig. 13 it shows a sinistrorse nemameric twist. Dotted x-points indicate the reverse side. Fig. 14. The genonema shown in Fig. 13 will form a helix as a tape wound around a pencil as a result of further expansion of the nemameres without the interference of external forces. This helix will be in the same direction as the nemameric twist, sinistrorse in the case of Figs. 13 and 14. This is one alternative of coiling. The other is described in Fig. 15. Fig. 15. A dextrorse spiral will result from the condition shown in Fig. 13 if an external force unwinds the nemameric spiral and orients all x-points toward one main direction in space. The peculiar arrangement of individual nemameres is due to the resultant of two forces acting on each of them—A, the external orienting force, pulling all x-points towards a plane below the helix, and B, the repulsive force between x-points and the following (trailing) edge of the next nemamere. This latter force causes all x-points to be further removed from the observer than the next trailing edge. Trailing edges or at least their lower ends show a relative right-shift away from the x-points. The resultant of these two forces on the genonema as a whole is in our case a dextrorse helix.

draw all x-point edges into one direction in space a nemameric twist would be transformed into a helix, thereby undoing the nemameric twist. This may or may not be accompanied by further (secondary) contraction of the thread. If the polarizing force is active immediately after the separating and rotational mechanisms come into play, the torque of the latter will be counteracted by the untwisting effect of the former just a few nemameres up the line and the genonema will split, twist and untwist and hence coil in scarcely separated events of a compound occurrence. Sister threads will coil in the same direction and hence separate readily. Gene multiplication could conceivably take place at any stage of the cycle, but nemameric separation is significantly linked to the beginning of a new coil. Likewise reversals of direction of coiling in a given piece of a gene string can only take place concomitantly with nemameric reproduction. In certain organisms straightening of the gene string may be very incomplete (*Tradescantia*), in others it may be complete. The scheme may be applied to meiosis as well as to mitosis. It likewise holds if nemameres produce their own mirror images instead of identical units. In this case genes and nemameres would be paired units at all times.

Contraction of the genonema may occur as follows: Completely relaxed, the thread is straight and there is no nemameric twist. Primary contraction at the time when daughter threads separate helps to establish the nemameric twist. Secondary contraction may enter in when the chromonema coils. Both primary and secondary contraction may be substituted for by varying the reactive forces of the x-points, allowing of chromonema elongation during prophase, in case cogent evidence to this effect should accumulate.

What in the above scheme is new, what is old, and what are the advantages of the scheme? The principle of the molecular coil, as suggested by Darlington, has been used to explain how basic units which are asymmetrically built may form a helix. The principle of the heterogonic sector

in a thread as suggested by Kuwada and Huskins and Smith has been used, inasmuch as one might look upon the x-points of the nemameres of the present scheme as projections of the heterogonic sector of the thread onto the units of its interior. The mode of separation of reduplicated genonemata and the randomness of direction of the nemameric twist as derived from the mode of reduplication as well as the integration of the principles mentioned are believed to be original. It appears desirable that further speculations on coiling may start from different premises and assumptions, since it is only too true that many assumptions now appearing justified may become obsolete in the near future.

In summary: It is speculatively suggested that the genonema is made up of nemameres represented by flat rectangular platelets carrying in one corner a characteristic called an x-point preventing rotational freedom over a small fraction of the arc. X-point edges separate last during nemamere reproduction. Torsional repulsion between daughter edges spreading from random points in both directions causes randomness of direction of the molecular coil now called nemameric twist. Helication occurs through orientation of subsequent x-point edges in one direction in space. The nemameric twist may thus be untwisted as fast as it is established.

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THE PHYSICOCHEMICAL NATURE OF THE CHROMOSOME AND THE GENE[1]

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1. THE FIBROUS NATURE OF THE CHROMOSOME

METAPHASE and anaphase chromosomes usually appear as fairly thick solid rods. Suitable methods of fixation and staining (particularly pre-treatment with acids or ammonia vapor) reveal more structure. The chromosome consists of the chromonema thread, which in mitosis is coiled in a single tight spiral, while in meiosis this spiral may be coiled again in a second spiral. The mitotic spiral is known as the minor spiral and has typically about thirty turns. The spiral which is superposed on this in meiosis is the major spiral and has about five or six turns; it has so far only been described in plants.

Cytologists have not as yet by any means reached agreement as to the phenomena of coiling. The most important questions have been discussed by Nebel in another contribution to this symposium. The basic fact which remains perfectly clear is that, on the microscopic scale (i.e., structures with dimensions from a few hundreds to a few thousands of mμ), the chromosome is built of a fine fiber. A similar conclusion may be reached from a consideration of the connections between the genes. As is well known, the genes are joined together in a linear array and must therefore have two ends which can join up with neighboring genes. There usually do not appear to be more than two such ends; if there were, we should be able to get branched threads in which one gene was joined to three others. Branched chromosomes have indeed been described, both on genetical and cytological evidence. But

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in some cases in *Drosophila,* Muller and Offermann have shown by investigation of the salivary glands that the genetical evidence was at fault, and the cytological evidence in other forms seems to require further confirmation. At present it seems justifiable to conclude that the gene has only two ends which are capable of stable attachment. This may not be true of the centromere, at which branching of the chromosome seems to be possible.

These two ends can not be regarded as poles. That is to say, the gene is not in any sense like a little magnet with different north and south ends. This is clearly shown by the fact that they can join up in reverse order in inversions.

2. The Size of the Gene and the Chromonema

Various attempts have been made to estimate the size of the gene. It is of course by no means clear that a gene has a definite and constant size; if the gene is a compound structure, it might grow gradually between two divisions. But the estimates of gene size are all rather rough at present, and it is very unlikely that the size of the gene is sufficiently variable to affect the order of magnitude. The estimates of size all aim at fixing maximum possible sizes; they show that, according to some particular line of reasoning, the gene or chromonema can not exceed certain dimensions. Clearly the most important datum will be the least maximum estimate.

All estimates of the dimensions of the gene start from considerations of the observed size of chromosomes or parts of chromosomes. Chromosomes certainly change in apparent dimensions during the processes of growth and division. Many of these variations can be explained as results of the changing degree of spiralization of the chromonema, and the relation between the gene and the chrosome, and thus the estimates of the size of the latter, always depends on the structure which we find or assume for the chromosome thread. The most securely founded of all statements about the condition of the chromonema is probably that it is completely straight and uncoiled in

salivary gland chromosomes. An estimate of the length of the chromonema associated with a gene can be made on this assumption. Muller and Prokofieva studied seven chromosome breaks occurring in a short region at the left end of the X chromosome in *D. melanogaster*. They found that the breaks apparently occurred in only four different places; and they concluded that these four places were the connections between five successive genes in the linear sequence. Further, they obtained a case of a minute deficiency which was viable in a homozygous condition and showed a phenotype indicating the absence of only two genes, yellow and achaete. By cytological examination of the salivary gland chromosomes in these cases they deduced the interval between successive genes to be about 125 mμ; the yellow achaete deficiency was just visible as the loss of a part of a dark band.

Estimates of the widths of the genes can not be obtained directly from measurements of the widths of the chromosomes. In salivary glands, the individual chromonemata can not be distinguished, being presumably below the resolving power of the microscope. By ultra-violet light, at least 64 parallel threads can be seen in *Drosophila*, and up to 400 in the thicker chromosomes of *Chironomus;* there may be even more. In other stages, the apparent thickness is dependent partly on the spiralization and perhaps also on the amount of nucleic acid condensed on the thread. Muller has made an estimate of the maximum thickness of the chromonema and therefore presumably of the gene by assuming that when the chromosomes are most contracted at meiotic metaphase they are entirely filled with the coiled-up thread. The active part of the X in *D. melanogaster* has a volume of about 1/12 cu. μ. The length of the thread, when uncoiled, is found to be about 200 μ in the salivary glands. If the thread has a square cross section with a side x, we can determine x, on the above assumptions, from the relation 200. $x^2 = 1/12$, whence $x = 20$ mμ.

This again is a maximum estimate, since there is no proof that the chromonema does fill the whole of the meta-

phase chromosome, part of which may be occupied by accessory material. Cases are known in which the dimensions of metaphase chromosomes are under genetic control; for example, in some species hybrids, chromosomes may appear up to ten times larger than they normally do, and this increase is almost certainly due to the accretion of extra chromatic material on to their surface. Moreover, it is probably that the inert regions of the chromosomes are not fully spiralized at metaphase, yet they appear, with most stains, to be of the same thickness as the active parts; this thickness must be due to the condensation of material on to the surface. There are then considerable grounds for supposing that a metaphase chromosome contains other material in addition to the chromonema, and that the estimate of the thickness of the chromonema given above is considerably too large.

Darlington has arrived at an estimate of volume by a method somewhat similar to Muller's; dividing the number of bands discernible in the IIIrd chromosome in the salivary glands of Drosophila (say 2,000) into the volume of the chromosome at mitotic metaphase, (say 6×10^7 cu. mμ) he finds the volume of the gene to be 3×10^4 cu. mμ, which agrees fairly well with Muller's estimates given above.

The two best estimates we have obtained above are maximum estimates for the dimensions of parts of the chromonema; in the first place a length of about 125 mμ seems to be associated with each gene, and secondly the maximum thickness of the thread is about 20 mμ. If we can accept these two estimates, arrived at in different ways, as of about equal accuracy, it is perhaps significant that even the units of the chromonema are elongated, fiber-like bodies. But we must note that, quite apart from the possibility that these figures may overestimate the size of the chromonema-unit-particle, there is no proof that the whole of this particle is occupied by the gene. It is possible to assume that the gene is a very much more minute body embedded within and always associated with the larger particle whose size we have been estimating. We shall discuss this possibility further on.

3. The Chemical Nature of Chromosomes

Chromosomes as such have never been chemically analyzed; they are too small for present methods. The nearest material which can be collected in quantities large enough for ordinary chemical investigation is sperm, particularly fish sperm. The head part of the sperm consists almost entirely of nuclear material, and analyses of this show that the two main constituents are thymonucleic acid (about 60 per cent.) and simple proteins of the kind known as protamines (35 per cent.). Nucleic acid combines very easily with proteins to form complex nucleoproteins, and it probably occurs in this combined form in the nucleus.

The distribution of nucleic acid in the nucleus can be investigated by means of ultra-violet spectroscopy, since it has a characteristic strong absorption at wave-lengths near 2,600 Å. During division stages, when the chromosomes are contracted and can be seen as separate bodies, almost all the nucleic acid is attached to the chromosomes; it may also be in the chromosomes in resting stages, but the fully extended chromonemata are not separately distinguishable from the nuclear sap, and the evidence is therefore not clear. The metaphase chromosomes can also be shown to contain protein, since they are attacked by proteolytic enzymes. In the salivary gland nuclei, the chromosomes contain the same two constituents, which perhaps makes it likely that the resting stage chromosomes are built up in the same way, and that the chromosome constitution is constant throughout the division cycle. Recently, however, Koltzoff has claimed that there is one stage in the development of the so-called "lampbrush" chromosomes in amphibian oocytes in which they contain no nucleic acid.

The chemical make-up of protein is not yet fully understood. It is known that some of the chemically rather inert proteins such as hair and silk are formed from fibrous elements consisting of chains of "polypeptide links," each link having the constitution–CO–CHR–NH–, where

R is a group (the side chain) which may be a simple hydrocarbon, an alcohol or a base such as arginine. In the fibrous proteins just mentioned, the links are arranged in linear chains, the chains being connected together by means of the side chains. There are, however, other types of proteins in which the molecules seem to be spherical rather than elongated; these are known as the globular proteins, and there are others intermediate between the fibrous and globular types. We do not know to which of these types the chromosome proteins belong, since the protein isolated from sperm (clupein) has not been examined from this point of view. The thread-like appearance of the extended chromosome, and the two-ended nature of the chromomeres, suggests that the chromosome consists of protein fibers arranged more or less parallel to its length. But this does not by any means necessitate the assumption that the chromosome protein is itself fibrous, since the orders of magnitude are quite different. The polypeptide links in a fibrous protein chain are about 0,35 mμ long by 0,45 mμ thick by 1 mμ wide in the direction of the side chains. The visible chromonema is a few hundred mμ thick, while Muller's estimate, based on its length in salivary gland chromosomes, gives it a width of about 20 mμ. Fibers as large as this can just as well be formed from globular as from strictly fibrous proteins, since the units (molecules or repeat cells in crystals) of the former are about 6 mμ in diameter, and cases are known, for instance, in some of the virus proteins, in which these units unite to form fibers a few tens of mμ thick. Studies on the extensibility, and particularly the reversible extensibility, of the chromosomes give some, and could probably give much more, information about globular or fibrous nature of the chromosome proteins. In completely fibrous proteins the polypeptide chains lie fairly parallel and are more or less unfolded; they can only be stretched by actual straining of the chemical bonds. It is probable, however, that in globular proteins, the same or very similar polypeptide chains exist in a folded configuration, so that ex-

tension of fiber constructed of globular protein involves only the unfolding of the chains and can proceed much further before the fiber is ruptured. Duryee has shown that the lampbrush chromosomes of amphibian oocytes can be reversibly extended to about 3½ times their normal length, at least under favorable conditions (in absence of calcium or other heavy metallic ions). Salivary gland chromosomes easily stretch at least to twice their length and probably can be stretched further when special efforts are made to do so by microdissection methods. Thus even in chromosomes in which the chromosome thread or chromonema is apparently uncoiled, the thread itself has considerable elasticity, and perhaps may be constructed of globular proteins in which the polypeptide chains are folded on a molecular scale. Much further study is required, however, before this can be taken as more than a suggestion.

When we turn to consider the other main constituent of chromosomes, the nucleic acid, a series of facts emerge which are extremely suggestive of an essential connection between nucleic acid and proteins, but whose exact significance can not yet be stated. Nucleic acid itself easily forms fibers and x-ray studies have shown that these consist of a chain of phosphoric acid residues to the side of which are attached a series of flat, plate-shaped groups each of which contains a purine base attached to a sugar. The first remarkable fact is that the repeat distance along the chain, *i.e.*, the distance between neighboring phosphoric acid residues, is almost exactly the same as the repeat distance in a polypeptide chain; 0,336 for the nucleic acid, 0,334 mμ for the polypeptide. The difference, which may not be significant, is at least so small that it is easy to imagine that the polypeptide and nucleic acid chains might unite parallel to one another to give protein-nucleate chains. This can in fact actually be observed; Astbury has prepared the nucleate of clupein, the protein isolated from fish sperm, and shown that it is a fibrous material. Further confirmation comes from a study of the double re-

fraction. Protein fibers have a somewhat weak double refraction which is positive in the direction of the fiber, while nucleic acid, in which there are large flat plate-like groups sticking out at right angles to the length of the fibers, has a much stronger double refraction which is negative in the direction of the fiber axis. The clupein-nucleate shows a double refraction negative in the fiber direction due to the nucleic acid. So do fully uncoiled chromosomes, such as those of salivary glands and zygotene stages; when the chromonema is presumably coiled in a single coil (*e.g.,* mitotic metaphase) so that it runs perpendicularly to the length of the chromosome, the sign of the double refraction changes and becomes positive in the direction of the *chromosome* axis, while in meiotic metaphase, where the minor spiral is coiled again in a major spiral, the double refraction reverses again and becomes once more negative in the direction of the chromosome axis. All these data fit in very well with the idea that the protein and nucleic acid have combined to form composite fibers in which the two constituent fibers lie parallel to one another.

The cytological evidence makes it quite clear that the chromosomes are not homogeneous structures. In the first place, there is a differentiation in salivary gland chromosomes between the darkly stained bands and the non-stained inter-band regions. The property of stainability depends on the content of nucleic acid, and the concentration of this substance in the bands can be demonstrated directly by studies of ultra-violet absorption. One must suppose that the proteins in the band regions have a particular affinity for nucleic acid, and Wrinch has suggested that this may be due to a higher concentration of basic groups, particularly arginine, which is known to be present in remarkably high amounts in clupein. The difference between the bands and inter-bands appears, however, rather larger and more sharply defined than would be expected if it were due to a merely quantitative difference, but this appearance may turn out to be illusory when

actual measurements of nucleic acid content become available.

The extensibility of the bands seems to differ sharply from that of the interbands, the former being much the more rigid. There are two factors to be taken into consideration here. Firstly, the nucleic acid fiber itself appears to be inelastic, and the rigidity of the bands may be due simply to their nucleic acid content. Secondly, while it is easy to see how nucleic acid may combine with fully extended polypeptides, it is not so clear how it can fit on to a globular protein; it is possible then that the proteins of the bands, when combined with nucleic acid, are in the extended form, and thus have themselves lost much of their extensibility; but whether this should be regarded as a result or as a contributory cause of their affinity for nucleic acid is as yet quite unknown.

The differential staining behavior of heterochromatic regions presumably depends on a chemical composition or physical state different to that of the euchromatin, but in general very little is known about this. In salivary glands of *Drosophila,* the inert regions show a structure of longitudinal striations and transverse bands which is somewhat similar to that of the euchromatic regions, except that the bands are more feebly staining and the whole structure less clear-cut. Although the inert region of X chromosome, for instance, is fairly short in the salivary gland chromosomes and has only a small number of bands, it occupies a large proportion of the whole chromosome at metaphase of mitosis. This may perhaps be partly due to a lesser degree of spiralization in mitosis, although, since the region at that time is definitely shorter than it is in salivary chromosomes, some spiralization must occur. It is probable that the large relative volume of the inert region in mitosis is at least partly due to an abnormally large concentration of nucleic acid on to it at this stage. Muller claims that the greater bulk of the mitotic inert region is produced under the influence of only two loci in

the region, and it is conceivable that the region contains loci specially concerned with the synthesis of nucleic acid.

The physical and chemical basis of this structure is unknown, but it is remarkable to find that the conditions underlying it appear to be transmissible; when inert regions are brought, by translocation, into contact with euchromatic parts of the chromosomes, there is a tendency for the latter to be modified in their appearance in salivary glands, so as to assume more nearly the inert structure. This suggests that the inert regions differ from the euchromatic regions only in some general condition which overlies the same basic differentiation into band and interband.

One physical variable which comes to mind in this connection, but about which we unfortunately know much too little, is the water content. It seems plausible to suggest that changes in hydration of the chromosome proteins occur during the mitotic cycle, perhaps correlated with the hydration changes which are suspected to occur in the proteins of the spindle. Reversible hydration and dehydration of living salivary gland chromosomes has recently been observed under the influence of asphyxiation and changes in osmotic pressure. There are no data known to me as to regional differences in hydration at different parts of the chromosome.

The centromeres are probably quite differently constituted from the rest of the chromosome. They seem to be unable to transmit torsional stresses, since the directions of coiling at metaphase are apparently independent of one another in the two arms of a chromosome with a central centromere; and similarly interference in crossing over does not extend across a centromere. It has also been shown that when centromeres divide at metaphase they do not always split along a plane parallel to the length of the chromosome, but may occasionally be divided transversely or at any angle. All these facts tend to suggest that the centromeres, unlike the rest of the chromosome, are not fibrous structures.

4. The Nature of the Gene

In attempting to work out an adequate picture of the relation between the gene and the chromosome, one can start from the fundamental fact that the chromosome is an elongated structure which, whenever we can analyze it, has differences arranged in a linear order along it; these differences can be detected by linkage studies, chromomere structures, etc. The units, between which differences are noted, may be of different sizes according to the different methods of investigation; there are, in roughly descending order, inert or precociously condensing regions, large chromomeres, ultimate chromomeres or salivary gland chromomeres, and the units of cross-over and x-ray breakage. One might symbolically represent the chromosome thus: abcdefghIJKLMnopqRSTU'V'W', where there are differences on three scales, between the capitals and lower case letters; underlined, crossed and dashed letters; and finally the letters themselves. The smallest units in this scheme, symbolized by the individual letters, are the units of crossing over and x-ray breakage, and probably measure, as we have seen, about 100 mμ in length.

If we view the chromosome as it were through the other end of the telescope, attempting to build it up from chemical units, we arrive at a somewhat similar scheme of a linear order of units of different orders of magnitude. The ultimate units now are the links in a polypeptide chain, with a length of only 0,334 mμ. Exactly what the larger units are is more doubtful, but we have a range of possibilities; there are the periodicities along the chains, the repeat units out of which protein crystals are built, the protein molecules such as they exist in solution, and finally virus particles, all of which may be considered as providing suggestions as to the kinds of units which may be involved. These units range in size nearly up to 100 mμ which we took as an estimate of the smallest units to be considered when we approach the chromosome structure from the other end. It is, then, possible to conceive of the chromosome as a linear array of units, the units them-

selves forming a hierarchy all the way from heterochromatic and euchromatic regions, some tens of thousands of mμ long, to polypeptide links only a few tenths of a mμ long.

This apparent homogeneity in the type of formal order exemplified by the chromosome on different scales should not tempt one to suppose that other properties may be just as easily conceived of in any of these scales. For instance, it is sometimes suggested that because the nature of one link in a polypeptide chain may chemically affect the properties of a neighboring link, the same type mechanism may explain the phenomenon of position effect. But in the latter case, the influence is between neighboring genes (*i.e.* breakage units) and extends over distances about a thousand times as great as in the former case. No direct analogy between mechanisms of the two phenomena is possible; and in fact no example of a direct chemical influence extending throughout such a distance appears to be known in protein chemistry.

TABLE OF SIZES*

Vaccinia virus	175
Rous sarcoma virus	100
Tobacco mosaic virus	430 × 12, 3 × 12, 3
Bushy stunt virus	28
Haemocyanin molecule	59 × 13, 2 × 13, 2
S13 molecule	10 (? shape)
Repeat unit of virus crystal	15 × 15 × 7
Haemoglobin molecule	2, 8 × 0, 6 × 0, 6
Protein fibre (repeat unit)	0, 334 × 0, 45 × 1, 0
Nucleic acid (repeat along fibre)	0, 336
Gene (estimated maximum dimensions)	100 × 20 × 20
Sensitive volumes: gene mutations (Timoffeff-Ressovsky)	c. 1
gene mutations somatic, (Haskins and Enzmann)	15
cytological effects (Marshak)	5

* The sizes are given in mμ (= 10° A = 10^{-6} μ.) Where only one dimension is given, it is the diameter of a spherical unit. (Partly after Stanley.)

Certain of the properties of the genes give some hint as to the possible kinds of units which may fill the gap between the 0,334 mμ polypeptide links and the 100 mμ genes. The most important is the property of identical reproduc-

tion. Between two cell divisions, each gene causes the formation of another gene exactly like it; if the gene mutates into an abnormal form, it is the mutated gene which is reduplicated. The gene, then, must in some way act as a model on which the new gene is formed. This can only occur if chemical forces originating in the radicals in the gene can extend far enough to influence the nature of radicals formed in the equivalent places in the new gene. The thickness which we can postulate for the gene is therefore limited by the distance through which we can imagine such chemical forces extending. Probably the maximum estimate which is chemically reasonable is about 10 mμ, which is the order of magnitude of the thickness of the repeat units out of which protein crystals are built. This is of the same order of magnitude as the estimate given above for the maximum thickness of the chromosome thread. It is therefore impossible to reject, from consideration of gene reduplication, the idea that the gene is a single unit. On the other hand, a further difficulty arises in this connection, namely, the necessity to find some mechanism which accounts for the fact that only two genes, the old one and the new, are present at the end of each intermitotic period. The reduplication occurs only once. No plausible hypothesis to account for this has been put forward.

Alternatively, we may assume that the gene is compound, consisting of a number of identical sub-units. Such a supposition probably simplifies the task of accounting for gene reproduction. The chemical forces on which the identity of the new and old gene depend would not have to extend so far from the radicals to which they were due, since the thickness of the sub-units would be less than that estimated for the whole chromosome thread. Similarly, the reproduction might continue gradually, and the gene grow until it eventually split into two by reason of some instability which increased with increasing size, such as that which causes a drop to break up when it passes a certain size limit. The difficulty with this hypothesis is

the fact that some genes (though only a few) show more or less equal rates of back and forward mutation.

It appears not unlikely that nucleic acid plays some important role in the process of gene reduplication. For instance, the most rapid synthesis of nucleic acid occurs just before the prophase of mitosis, at the time when the chromosome appears to split or reduplicate. Again, it is remarkable that the virus proteins which share with the genes the property of identical reproduction in living systems, and of mutation, also contain large quantities of nucleic acid. Conceivably there is some connection here with the remarkable fact recently revealed by Schultz and Caspersson, that nucleic acid is in some way connected with the stability of the gene; when parts of the inert region in Drosophila are translocated into the euchromatic regions, they frequently cause the neighboring loci to become unstable and undergo somatic mutations which give rise to phenotypic spotting such as that found with other mutable genes; and this instability appears to be correlated with an increase in the nucleic acid content of the corresponding bands in the salivary gland chromosomes.

All the above considerations apply to genes considered as units of crossing over and x-ray breakage. It is quite possible that only a small part of the gene defined in this way is actually active in the control of development. We cannot rule out the possibility that this activity is due to some particular group within the large protein-nucleic acid complex we have been discussing. In fact the small size of the "sensitive volumes" found for particular steps of mutation might suggest that only quite restricted regions are concerned in producing the phenotypic differences between two allelomorphs; but it is well known that there are many uncertainties in the interpretation of the sensitive volume measurements.

On the other hand, it is quite possible that all primary gene products are enzymes and therefore probably proteins, which may be similar in composition to the genes themselves. It would then be in order to suggest a con-

nection between gene activity, in which enzymes were produced and liberated into cytoplasm, and gene reproduction, in which similar bodies were formed but retained in the neighborhood of the chromosome.

It will be apparent from the above discussion that the exact knowledge at our disposal is so meager that very many alternative hypotheses are still possible as to the nature of the chromosome, and the gene in its different senses. However, the enormously important effects of the genes on development, their capacity for identical reproduction, and the fact that they, rather than the cells of an earlier time, seem to be the most ultimate units into which we can analyze living organisms, makes the problem of their constitution one of the most fundamental questions of biochemistry, well worthy of discussion even long before it can be fully answered.

THE STRUCTURE OF SALIVARY GLAND CHROMOSOMES[1]

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THE present discussion is limited to the euchromatic regions of salivary gland chromosomes and will center about two main subjects. First, because the bands of the giant chromosomes have been shown to be associated with, or to represent directly or indirectly, the genes, a clear understanding of the nature of the elements which go to make up these chromosomes and their bands is a matter of fundamental importance for any study of the physical nature of the hereditary units. The second aspect, closely tied to the first, is the relation between salivary chromosomes and those we see ordinarily in mitosis or meiosis. With these topics in mind, we shall not consider earlier ideas about the origin and structure of salivary chromosomes. In this rapidly developing phase of cytology, with so many workers from diverse fields taking part, it has been found necessary to discard or greatly modify some of the original concepts, and a discussion of these changes, or a consideration of many special details would serve little purpose here.

The first step towards a clearer understanding of the nature of salivary chromosomes was made by Koltzoff and Bridges, independently, when they attempted to account for the great size of these elements in the fruit-fly. They suggested that the giant chromosomes represent uncoiled prophase chromosomes which have undergone a number of longitudinal divisions without an accompanying cleavage of the nucleus or the cytosome. In effect, each salivary chromosome is a bundle of chromonemata; the bands are transverse discs or, as they actually appear, rows of

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homologous chromomeres, of which 16 are commonly seen in *D. melanogaster,* and the achromatic spaces between the bands contain the thin strands or threads which connect the chromomeres of the separate chromonemata. We now realize that the size of the chromosomes in the fruit-fly is not satisfactorily accounted for on the assumption of two divisions of the four original chromatids, nor can we regard the 16 strands as univalent chromonemata or single gene strings. Nevertheless, later studies on more favorable material, such as Bauer's observations on Chironomus, or the work which Mr. Griffen and I have done on Simulium larvae, have demonstrated the essential correctness of the multiple strand concept. Were the direct evidence less convincing than it is, we would be forced to this conclusion by recent researches on developing insect tissues which show the common occurrence of multiple strand or polytene chromosomes.

In the fruit-fly direct evidence for multiple strands is usually restricted to the dash- or dot-like bands in which the separate chromomeres can be easily seen and counted. Only occasionally, and then in stretched areas, can one observe the very fine threads which connect the chromomeres in a linear series. But in species with broader and more loosely synapsed elements, such as Simulium, we generally get a clear picture of the structure. Fig. 1 shows the details seen at the surface of a short section of a salivary chromosome in *S. virgatum* after fixing and staining with aceto-carmine. Essentially the same features are seen at all optical levels as one focuses through the chromosome. Each band is made up of one or more rows of chromomeres, which may be small, densely stained and separate, or large, vesiculate and more or less crowded in one transverse plane. In some rows, as at *b, d* or *h,* the chromatin surrounding each chromomere may be dense and more or less fused together, while in other rows (as at *c* or *e*) the chromatin is more flocculent or granular and the boundaries of the separate chromomeres more distinct. Within a given row the chromomeres are usually about the

same size, but occasionally one or more units will be larger than the rest.

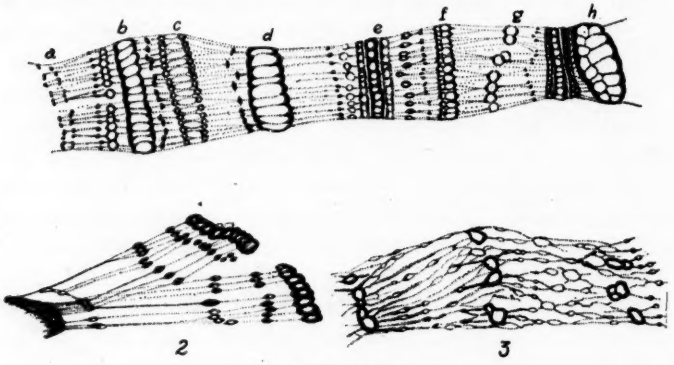
The threads which connect the chromomeres are very fine; they take little or no stain and are best observed in sharply oblique light. Since the four chromatids which go to make up these salivary chromosomes originally are twisted about each other, the fibers run slightly oblique to the longitudinal axis, but generally pass directly from the single chromomeres of one row to those of the next. In Simulium, however, not all the bands show the same number of chromomeres, some show about half as many units as their neighbors. In such areas (*d* in Fig. 1) the threads converge in pairs on the (double) chromomeres and diverge (as at *f*) when the chromomeres are again more numerous. The failure of the chromomeres of some bands to divide as often as others is specific, and shows even in the earliest ontogenetic stage.

Fig. 2, drawn at a higher magnification than the other figures, was made from an area where the chromosome was crushed and its elements separated. Here the multiple strand condition and the relation between the thread and its chromomere is very clearly shown. Notice particularly how some of the fibers stick together for a short distance and then separate and pass to single chromomeres. This is not the sort of behavior one would expect were we dealing with stress-lines, but fits in perfectly with the thread and chromomere concept.

The organization shown in Figs. 1 and 2 is typical for all portions of the Simulium chromosomes except a short area which we have called the "spread out" region (Fig. 3). Here the structure is more complex. The chromomeres are not arranged in compact bands but are more scattered and the threads connecting chromomeres of various valences form a network much as Metz has described for other forms. Were we to consider the structure of the spread-out region alone, we might well conclude with Dr. Metz that salivary chromosomes are alveolar in nature, but the development of this small area shows it

to be essentially similar to the more characteristic euchromatic portions shown in Figs. 1 and 2.

Turning now to the bands, a point I wish to stress is that many of them are compound in the sense that they are composed of two or more rows of different kinds of chromo-



FIGS. 1–3

Figs. 1 to 3 are from *S. virgatum*. Fig. 1 shows the details seen in a surface view of a fully differentiated salivary chromosome. The various bands, many of which are compound, are made up of discs of homologous ultimate chromomeres which appear as rows in any one plane. Some chromomeres are small densely staining and separate, others large, vesiculate and crowded. Fig. 2 shows a small portion of a salivary chromosome which was crushed in mounting. It is presented to show the relations between the longitudinal fibers and the chromomeres which they connect. Fig. 3 shows the typical structure of the "spread out" region. Here there are no definite bands and the fibers connecting chromomeres of different valences appear to form a spongy network.

meres closely associated linearly (Fig. 8). This feature has important implications and applications for cytogenetics and especially for Drosophila workers. In well-fixed and stained salivary chromosomes of Simulium, the different rows of chromomeres within a band show with considerable distinctness, but in forms with narrower chromosomes, such as *D. melanogaster*, the individual rows may not show, although many of the bands have been proved compound, both morphologically and genetically (*e.g.*, Muller, Ellenhorn and Prokofieva, 1935). Any one who tries to determine the exact pattern of a short area is apt

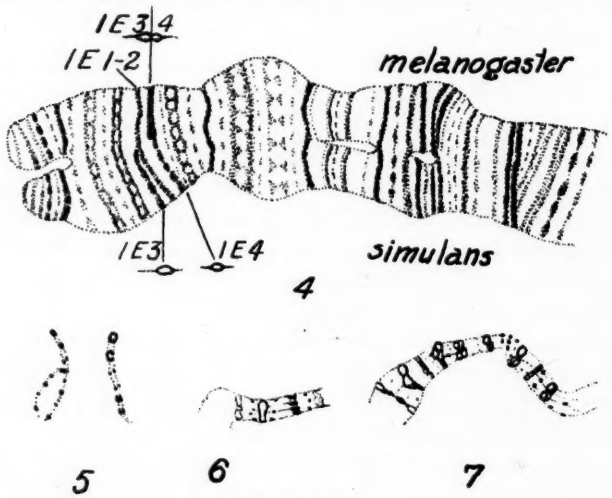
to find that the more a given region is stretched, the more bands become distinguishable. Of course the more a chromosome is stretched the greater the chance for the separation of these closely joined chromomere rows, and thus a broad band, in a lax chromosome, may resolve itself into a number of finer bands. Our present chromosome maps for *D. melanogaster* are incomplete and will not be entirely satisfactory until we know the chromomeric constitution of each complex band.

In locating gene loci, in estimating the size and the number of genes, especially in detecting small inversions or deletions, we must recognize the compound nature of many of the bands, and take into account the nature of the individual chromomeres which are involved. Let me illustrate: in hybrid larvae between *D. simulans* and *D. melanogaster,* one of our students, Mr. Horton, has found a tiny inversion in the X-chromosome involving one whole band and a part of another (Fig. 4). In *melanogaster,* the band 1E3–4 appears as a heavy double band. In *simulans* an inversion has divided this "doublet" into a broad and a narrow component. The obvious explanation is that the band 1E3–4 is made up of two rows, at least, of unequal sized chromomeres which were separated by the inversion, but there is no suggestion of this composition of the band in *D. melanogaster.*

A great deal of light has been thrown on the fundamental structure of the giant chromosomes by a study of the way they develop from small nuclei. Workers in the field are pretty generally agreed that the homologous chromosomes which unite to form the salivary chromosomes are split prior to somatic synapsis. Mr. Griffen and I have succeeded in tracing salivary chromosomes back to this four-strand stage. Each of these four chromatids shows the ordinary chromomeric structure of early prophase chromosomes (Fig. 5). The individual chromomeres are tiny, there isn't a great range of size, most of them stain solidly, but a few seem to show hollow or achromatic centers. The fiber which connects these chromo-

meres is extremely fine, it is more the pointed ends of the chromomeres and their linear order which indicates its presence.

The first step in the differentiation process is a growth of the individual chromomeres and not their visible division. As this goes on the sister chromatids and their homologues join in somatic synapsis. In four-strand chro-



FIGS. 4–7

Fig. 4 is taken from a hybrid larva of *D. melanogaster* and *D. simulans* and shows the left-hand end of the X-chromosome. A very small inversion in *simulans* has separated the band 1E3–4 into two components of unequal size showing that this band in *melanogaster* is compound and composed of at least two rows of unequal sized chromomeres. Figs. 5 to 7 are taken from *S. virgatum*. Fig. 5 shows the chromomeric structure of the chromatids from which the salivary chromosomes are formed. Figs. 6 and 7 are taken from 4 strand salivary chromosomes showing the increase in diameter which accompanies the growth of the individual chromomeres.

mosomes there is considerable difference in breadth, as Figs. 6 and 7 will show. As the chromomeres unite by somatic synapsis to form the incipient bands, more and more details become visible. This is due, in some part, to the increase in size which makes the components more easily seen, but a considerable part is due to the spreading

out, probably an uncoiling of the ultimate chromomeres contained within the compound chromomeres of the chromatids which we see earlier. Somatic synapsis seems coincident with the separation of the ultimate chromomeres.

The salivary chromosomes increase rapidly in breadth, and as broader elements are observed we see more chromomeres in the discs or rows and more fibers in the interband area. It is clear that the original chromatids divide and in the end each of the four chromatids we start with contains on the average some 16 strands, making a total of 64 strands per (paired) chromosome. This means that each of the original chromatids has undergone four divisions, a fact that will not account for the great breadth or length of the adult chromosomes. As I have emphasized, it is the hypertrophy of the individual chromomeres which accounts for most of the increase in diameter. Not all the interesting details of development have been worked out yet in Simulium, but it is perfectly clear that we start with four chromatids or strands which are chromomeric in nature and end with some 64 strands, each of which is chromomeric in character.

As the salivary chromosomes grow in diameter the shapes of the chromomeres may change in crowded bands. The chromatic hull is the part most affected and in Fig. 8 the sequellae of this crowding process is shown diagrammatically, for units which have a dense rind of chromatin. At the outset, to the left in Fig. 8, the chromatin is more or less evenly distributed about the vesicle. But as the chromomeres become crowded, the chromatin is shifted to the free ends where it unites apparently into a transverse plate. The extent of the shifting of the chromatin depends on the size of the chromomeric vesicles, and thus the appearance of a given band may be quite different in a number of slides. These transverse plates of chromatin have caused Dr. Metz to envision salivary chromosomes as a series of alveoli separated by chromatin discs, a concept which is reasonable enough, if we confine our attention to

fully developed chromosomes, but patently untenable, in view of the developmental history of the salivary chromosomes, if Mr. Griffen and I have correctly recorded it.

Of especial interest, in Fig. 8, is the middle diagram which shows the behavior of the chromatin when homologous chromomeres unite into clusters rather than into transverse rows. Just as in bands, the chromatin tends to lie on the surface of the mass and the boundaries between the individual chromomeres of the ball soon disappear. This rounding up must involve some slipping of the chromomeres so that the enlarged structure is not made up of segments, like an orange, but of many displaced units. This point is of interest from two standpoints. First, it accounts for some of the increase in length of the fully differentiated chromosomes and more important, I think the same process goes on within all chromomeres, as explained below.

What is the nature of the hypertrophy of the chromomeres during the development of salivary chromosomes? To answer this question let us turn to a line of cytological investigation begun before the salivary chromosome era. In 1925 Jacobj showed that in the mouse liver the nuclear volumes do not give a bell-shaped curve, but a series of very sharp peaks, each peak corresponding to an exact doubling of nuclear volume. He interpreted his observations to mean that there had been an "inner division" of the chromosomes without nuclear cleavage as Heidenhain had assumed some years before. Using the same method of study, Hertwig measured the nuclei and computed the volumes of large nurse-cells and salivary gland cells in *D. melanogaster* and concluded that on the basis of nuclear volumes, the chromosomes in salivary glands should show at least 256 strands. This evidence stands in sharp contrast to the 16 strands which we usually see. In Chironomus, which presumably is much like *D. melanogaster* in the ranges of nuclear volumes, Bauer finds evidence for two hundred or more strands, which is a nearer approach to the 256 strands expected on the basis of Hertwig's esti-

mates. Buck has studied the increase of nuclear volume in Sciara and indicates that the order of increase is a thousand fold or more. I have not made a study of nuclear volumes in Simulium, but I have measured and computed the volumes of single early prophase chromomeres and

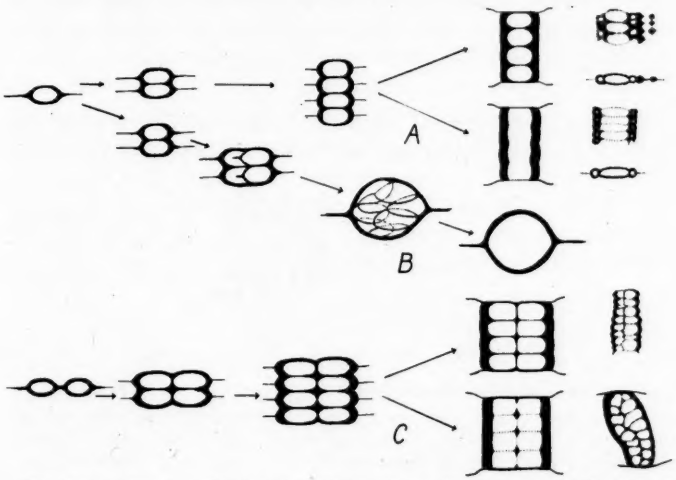


FIG. 8 is a diagram showing the effects of crowding on the distribution of the chromatin in bands when the individual chromomeres are covered by a dense hull of this material. A illustrates the formation of a "double band" both from a single row of chromomeres (on the left) and from three or four rows of non-homologous chromomeres (on the extreme right). B shows how extremely large chromomeres are formed in the "spread out" region. Not only is the chromatin squeezed to the surface of the cluster but there is also some displacement of the separate ultimate chromomeres. C illustrates the way chromatin is displaced when two non-homologous chromomeres are closely associated linearly, and at the right, actual examples of such compound bands taken from Simulium.

those seen in a fully developed salivary chromosome and find that the volumes of a single row of chromomeres, in old larvae, is sufficient to encompass 1,984 of the chromomeres seen at the earliest stage. In other words, each of the 64 chromomeres in Simulium is sufficiently large to contain 32 of the original chromomeres. It is not to be supposed that such measurements and hence volume calculations are very exact, but they all are more or less in line

and show that the chromomeres we see in fully developed salivary chromosomes of Simulium, and other species, must be compound and made up of a considerable number of homologous chromomeres. Whether the number is actually 32, as our measurements suggest for Simulium, or more or less, is not so important as the fact that we are not dealing with single ultimate chromomeres in Belling's sense in the salivary glands studied so far, but with aggregates of these.

If we are somewhat hesitant in accepting conclusions based on nuclear volumes, let us consider the work which

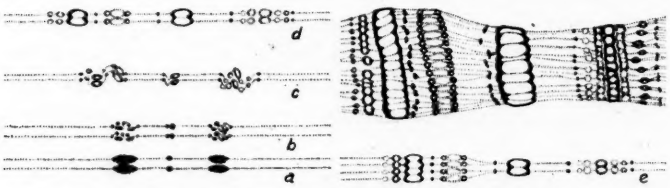


FIG. 9. This diagram illustrates the origin of a number of bands in salivary chromosomes from single compound chromomeres of an ordinary somatic chromosome. Only two chromatids are shown, each with three compound chromomeres (A). Within these compound chromomeres the ultimate chromomeres are arranged probably in a spiral (B). The ultimate chromomeres grow in size and uncoil, thus increasing the length of the salivary chromosome (C and D). Coincident with this somatic synapsis occurs (D). Later the original chromatids divide and ultimately there are an average of about sixteen strands in each of the four chromatids which go to make up salivary chromosomes.

has appeared recently which strikingly verifies the general correctness of our inferences. In the water strider, Gerris, we have enormous lobed nuclei in the salivary glands. Geitler (1937) has studied these. In the male, the X-chromosome remains condensed or heteropycnotic during the interphase of mitosis and by counting the number of heteropycnotic elements in a nucleus its chromosome valence, diploid, tetraploid, etc., can be determined. In the giant lobed salivary nuclei, Geitler finds great numbers of these heteropycnotic X's and while exact counts are impossible, he estimates that there must be around a thousand. Most of the larval cells studied so far in insects do not

divide, so that special interest centers about cells with high chromosome counts which can divide, as reported by Berger and by Bauer. The diploid number of chromosomes in the mosquito is 6, but Berger has observed in the mid- and hind-gut of larvae and pupae extremely large nuclei which often undergo division. The highest number of chromosomes which Berger had counted is 192. In the mitoses which follow during metamorphosis, Berger thinks that the chromosome number is reduced again so that in the adult cells nuclei with low valences are the rule. Apparently, Berger has unearthed an ingenious mechanism to hasten the process of metamorphosis and if the development of the mosquito is typical of other insects, we have a possible mechanism for somatic segregation and variegation, in general, without invoking somatic crossing-over.

Bauer has studied the nurse cells of some Diptera in which he finds that as the nucleus grows the chromosomes become polytene. Of especial interest is the fact that these polytene chromosomes unite into a haploid number of bundles which show a banded form. Later these bundles fall apart and many small chromosomes are formed presumably through the coiling of the single chromonemata.

In the light of the foregoing it must be clear that the increase in the size of the chromomeres, during the ontogeny of the giant chromosomes, is a real growth process during which the ultimate chromomeres, in Belling's sense, are reduplicated over and over without a visible subdivision of these units. Of this there can be no doubt, but I hasten to add that in old larvae, there is evidence for the differential swelling (or contraction) of specific chromomere rows to form spindle-shaped, puffed and other swollen areas, and hence in part the final size may be due also to the accumulation of accessory material.

If we grant that the chromomeres we see in later stages are compound, the same evidence forces us to conclude that the single fibers which we see are also compound to the same degree. Thus in Simulium we infer that each thread

is made up of some 32 strands. Even these bundles of 32 or more threads are just at the limit of visibility, and it goes without saying that the single univalent chromonemata would be far below the resolving power of our microscopes. In this way the criticism, based largely on molecular consideration, is met that a single gene string could probably not be seen.

There is nothing in the visible structure of the very fine threads between the bands which suggests morphological differentiations such as we might expect were the genes located here. The chromomeres, on the other hand, show a great variety of form, of size and of behavior, and the great constancy of these as well as the linear order, and the evidence from deletions, point to the chromomeres as the places along the chromosome where the genes are to be looked for. The evidence indicates that the chromomeres of salivary chromosomes are not single units and thus assuming that the chromomeres are genes we are not looking at single genes but at aggregates of them, plus, in some cases at least, accessory material.

In Simulium the chromomeres range in size from tiny dots lying just at the edge of visibility and thus having a diameter close to 0.2 μ up to huge globular masses 2 μ or more in long diameter. All the larger units are vesicular, and the chromatin appears to form a rind or hull about the achromatic center. As Caspersson (1935) and others have shown the chromatin is really nucleic acid while the center of each chromomere, as well as the thread, is protein.

How are salivary chromosomes to be compared to ordinary somatic and meiotic elements? In the accompanying Fig. 9 is expressed my concept of the relation between the fully developed salivary chromosomes and the earlier ontogenetic prophase stage. The essential feature is that the chromomeres seen in the chromatids initially are compound and thus give rise to several rows of the ultimate chromomeres which we see in the fully differentiated salivary chromosome. Within the early compound chromo-

meres there is reason to believe that the ultimate chromomeres are coiled, or at least are not in a linear order. As the individual ultimate chromomeres grow in size, the coils loosen up and spread out and coincident with this we have somatic synapsis of homologous chromomeres. The fully developed salivary chromosome, then, is not comparable to a mitotic prophase but rather to an early meiotic prophase, like the pachytene, but instead of four or eight chromatids, we have a much higher number of strands.

Few cytologists will question the compound nature of the chromomeres seen in somatic divisions simply because it has often been shown that the mitotic prophase elements are shorter and show fewer chromomeres than the meiotic prophase. That the internal arrangement of the ultimate chromomeres is a spiral is an inference based on indirect evidence, for we have been unable to detect it by direct observation. A linear orientation of the ultimate chromomeres seems precluded because there would be nothing to prevent somatic synapsis from occurring in any prophase stage. A spiral or irregular arrangement of the ultimate chromomeres within the compound chromomere would prevent the union of homologous units but would allow a general attraction of homologous regions. To my mind, however, the most cogent evidence of such localized minor coils comes from the field of genetics. Chromosome rearrangements, produced either by irradiation or occurring spontaneously, are predominantly small. For example, in hybrid larvae between *D. simulans* and *D. melanogaster*, of the 24 changes seen or deduced, only one is large; the rest are from 1½ to 10 easily seen bands. Needless to say, the intimate physical contact of the different ultimate chromomeres within the coils of these compound chromomeres would favor minute changes, on either the one or two hit hypothesis.

In presenting the foregoing concept of salivary chromosome structure, I must point out that Dr. Metz and some of his students interpret the features which we all see in fixed and stained preparations in quite a different way.

While he is quite willing to admit that there is probably a very high number of chromonemata in large salivary nuclei, Dr. Metz thinks that the "threads" we actually see are artifacts, stress-lines perhaps; and not bundles of chromonemata, as I would interpret them, nor does he recognize any genetic continuity between the "chromomeres" of fully developed salivary chromosomes and the chromomeres seen in the initial stages of salivary chromosome development. His views are founded on a study of living chromosomes, principally of Sciara and Chironomus, and on fixed and stained preparations of more or less fully differentiated salivary chromosomes. As yet he has not shown how these chromosomes develop. Adult structure, however, as we all know, is best understood in the light of ontogeny, and the fact that at the beginning, in Simulium, the chromatids consist of chromomeres connected by a fine thread and at the end of differentiation you still have visible threads and chromomeres is, to my mind, decisive proof for the concept of salivary chromosome structure which I have put forward.

In studying living chromosomes, however, Metz and his students, principally Buck and Boche, are bringing to light facts which promise to greatly extend our understanding of both salivary chromosomes and other types. I refer to the work which shows that living salivary chromosomes are extremely sensitive to changes in the density of the surrounding medium during which they decrease and increase in volume more than 50 per cent. without apparent injury. Such changes in volume may be accompanied by changes in optical properties, as Shinke (1937) has pointed out. Thus in Sciara, which normally shows little evidence of banding in the unaltered living state, the bands show clearly when the salivary gland is placed in salt solutions or in fixatives. Now it is well known that proteins show an extraordinary ability to absorb and give up water reversibly without changing in composition. Recently, for example, Bernol, Fankuchen and Riley (1938) show that crystals of the tomato Bushy Stunt virus swell about 80

per cent. on the addition of water and shrink again the same amount when water is withdrawn. The studies of Caspersson and others have shown that both the threads and the vesicular material of the chromomeres are protein, and the observations of Buck and Boche (1938) may be taken to indicate, in another manner, this same conclusion. In fact, it appears to me as extremely probable that in living salivary chromosomes the proteins must exist in a greatly hydrated state so that the chromomeres are very much larger, than in fixed nuclei, and the fibers swollen into columns of disperse protein micelles. On dehydration, either by the use of salt solution or by fixatives, the extraction of water would cause the protein micelles to lie closer together and when sufficiently contracted, to show as visible fibers. Stretching of the fixed chromosomes might act in a somewhat similar way and cause the more disperse micelle to form more visible aggregates. If I have correctly interpreted this general situation then we have an explanation for a puzzling feature of salivary chromosome structure. We know that the attraction between homologous chromomeres is very strong, so strong that the shape of the entire chromosome may be distorted in order for like units to synapse. And yet, within a chromosome, the very small chromomeres lie quite separate, in fixed preparations. What holds these small chromomeres apart? Obviously, if in the living state the fibers are really hydrated columns of protein, we have a mechanical set-up which would hold the smaller chromomeres apart. Of course, after fixation the attraction no longer exists.

By way of a summary, the salivary gland chromosomes are to be looked upon as bundles of entirely uncoiled chromonemata and thus are comparable to the chromosomes seen early in meiosis. They differ from other polytene chromosomes in the degree of uncoiling which permits the union of homologous ultimate chromomeres, or clusters of these, and other sequellae of somatic synapsis. The visible chromomeres and threads are not to be regarded as

single gene strings, but aggregates of these, the number varying with the age of the chromosome, ontogenetically speaking, and with the species. Nevertheless, the fundamental chromomeric nature of all chromosomes is clearly shown and the large size of the salivary chromosomes and their extended state make them the best material for the application of physical and chemical methods and concepts.

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CHROMOSOME STRUCTURE AS VIEWED BY A GENETICIST[1]

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I SHALL begin this presentation with the assumption that the A-B-C's of this problem are familiar to my audience and moreover that I am talking to a group who, although they may not be familiar with all the intricacies of our arguments, accept the validity of fundamental concepts developed by geneticists. This stand greatly simplifies my position, since it makes it unnecessary for me to discuss the evidence in support of it by now ancient and well-known postulates such as the evidence that genes are located in chromosomes, that they are arranged in a linear order, that homologous chromosomes may exchange sections through a process called crossing-over. This leaves me free to devote my time to the discussion of concepts which have recently evolved in this rapidly developing branch of biology; to concepts which are more intriguing since they have not yet passed the argumentative stage.

A geneticist is interested in chromosomes because they are carriers of genes and, therefore, his primary interest in the problem under discussion deals with the interrelationship between genes and a chromosome. One of the first questions which arises in connection with this problem is the relationship between the size of chromosomes and the number of genes they contain. Excellent evidence available from genetic and cytological studies of various species of Drosophila shows clearly that, as a rule, there is a close parallelism between the number of genes and the length of the chromosomes. In general, small chromosomes carry few genes, medium-sized ones carry more and

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long ones carry still more genes. However, as usual, there are exceptions to every rule. Chromosomes are known which are long in size but at the same time which carry very few genes or no genes at all. Well-analyzed cases of that type are the Y-chromosomes of various species of Drosophila. In *D. melanogaster,* for example, the metaphase Y is longer than the X, but X carries almost one hundred times as many known genes as Y. The fact that the relative lengths of these two chromosomes are not the same in all tissues indicates that this is an unusual situation. In salivary glands, for example, the length of Y is only one fiftieth the length of the X, and also the consistency of Y is different from that of the other chromosomes. The whole Y-chromosome, as well as certain regions of some other chromosomes adjacent to the spindle fiber, are heterochromatic. The remaining sections of the chromosomes are euchromatic. In salivary glands euchromatic regions show distinct banding, while heterochromatic sections have loose and not well-defined structure.

Bauer, Demerec and Kaufmann (1938) have found that breaks induced by x-rays are distributed at random within euchromatic sections of salivary gland chromosomes of *D. melanogaster.* They have found also that in heterochromatic regions breaks are more frequent than in euchromatic regions for similar salivary chromosome lengths. However, studies of Kaufmann and Demerec (1937) indicate that breaks in the entirely heterochromatic Y-chromosome occur with approximately the same frequency as in the euchromatic segments of autosomes of equal length if this length is determined on the mitotic metaphase chromosomes rather than on salivary gland chromosomes. From this evidence Kaufmann and Demerec concluded that the metaphase chromosomes approximate more closely than salivary chromosomes the proportional chromonema length of the two regions, and that the frequency of breaks per unit is the same in both. This suggests that the structure of the chromonema is similar in both regions, which is equivalent to saying that this structure is similar

throughout the whole chromosomal complex. This opinion is opposed by Muller and Gershenson (1935), who think that breaks in heterochromatic regions are not distributed at random but are localized to certain places where they occur with higher frequency than in euchromatic regions. They conclude that the heterochromatic regions of X and Y are fundamentally different in structure from the euchromatic regions, and that they consist essentially of non-genic material derived from a very few specific active genes, between which breakage takes place much more readily than between genes in the chromonema of the euchromatic regions. Recent cytogenetic studies on distribution of breaks in the Y-chromosome made by Neuhaus (1938) as well as cytological studies carried on by Kaufmann (unpublished) support the observations of Kaufmann and Demerec (1937), namely, that breaks in the heterochromatic Y-chromosome are distributed at random. These observations are in favor of the assumption that the frequency of breaks per unit of chromonema is similar in all regions of the chromosomes.

Chromosomes, then, may be visualized as composed of two components: (1) of the fiber-like chromonemata which are structurally similar throughout the whole chromosomal complex, and (2) of the material linked to the chromonemata which differentiates various sectors of a chromosome into units called genes by geneticists. This picture has its close counterpart in the picture of the fiber protein molecule as outlined by Astbury and Bell (1938) and discussed here to-day by Waddington (1939). The backbone of the protein molecule may correspond to the fundamental unit of a chromonema, and a group of radicals attached to the backbone may correspond to what we call a gene.

It is of interest to note that the evidence is accumulating which indicates that radicals rich in nucleic acid are associated with the genetically active regions of chromosomes. This is suggested by direct cytological observations of salivary gland chromosomes where chromatin bands are found to be closely connected with genetic loci, and also by

the results obtained with ultra-violet radiation where it was found that the region of the spectrum absorbed by the nucleic acid is the most effective in producing what may be interpreted as genetic changes (Hollaender and Emmons, 1939).

By the use of x-rays it is possible to induce various changes in genes. Many of these changes are lethals, and for some of them it is possible to establish through salivary gland chromosome analysis that they are deficiencies. In some cases this analysis was carried to very small sections, probably to single loci. A study of such deficiencies shows that not all regions of a chromosome are of equal importance to the organism (Demerec, 1935, 1938). It has been found that deficiencies for certain loci are not only lethal to the organism as a whole but that they are lethal even to a small group of cells which would develop as a mosaic patch within the normal tissue (Demerec, 1934; Slizynska, 1938). On the other hand, chromosome regions are known where a deficiency has no detectable influence on the organism (Demerec and Hoover, 1936; Bridges, 1938). Between these two extremes several intermediate stages were detected. For example, deficiencies are known which are lethal to the whole organism but are not lethal to a small group of cells (Demerec, 1934), and some which are not lethal to the organism but manifest themselves as mutant characters (Muller, 1935). That a situation similar to this observed in *Drosophila melanogaster* probably exists in other organisms as well is indicated by results obtained by McClintock (1938), who, in her work with maize, found that the effect on viability of certain deficiencies is not correlated with the length of the deficient segment but rather with the region which is deficient.

What may be responsible for these differences between various loci? It seems probable that new loci originate through the duplication and subsequent differentiation of existing ones. A number of such duplications, which are viable in the homozygote, are on record and they might well be the progenitors of new loci. The most illustrative

case is the Bar duplication cytologically analyzed by Bridges (1936). A good example for the mechanism of origin of duplications is the case of x-ray-induced duplication in tandem described by Kaufmann and Bate (1938). If a small section of a chromosome is duplicated the loci of that section would be present twice and any change or deficiency affecting one gene located in that section would not be expected to show. This would give greater possibility for changes in duplicated genes, since the otherwise detrimental effect of such changes would be neutralized. Also because of the new position of the duplicated section position effect might enter into play and stimulate the differentiation of the genes involved. In the course of time, therefore, duplicated genes would differentiate into new loci which would be essential for the balance of that particular gene system. However, before this final step in the differentiation process is reached intermediate steps would have to be passed. Consequently, in a system where such a process is continuously at work it is to be expected that all stages of differentiation of loci would be represented. In such a case, the cell-lethal loci would be entirely differentiated loci not protected at all by duplications, while the loci with viable deficiencies would be those which are fully protected. The other groups would represent intermediate stages.

One would therefore expect to find an inherent interdependence between genes of a gene system even after they have become differentiated far enough so that their presence is essential for the existence of that system. There is ample evidence available indicating that such interrelationship does exist. The most conclusive evidence of this is the fact that the balance within a gene system is so sensitive that the absence of even one gene out of a total of several thousands may upset it to such an extent that this system is not able to function and the organism does not survive. Moreover, numerous cases of interaction between genes are on record where a change in one gene affects the functioning of another seemingly unrelated

gene. Another striking example of the interdependence of genes within a gene system is available in cases where the stability (mutability) of certain genes is affected by certain other genes. The mutability of the unstable miniature of *D. virilis,* for example, is greatly increased by the presence of any one of the four known genes none of which is located in the same chromosome with miniature (Demerec, 1929, 1930). Rhoades (1938) has found a gene in maize which causes another, otherwise stable gene, to become unstable. Finally Demerec (1937) has described a gene which appears to increase the mutability rate of the whole gene system.

There is ample evidence which indicates that the activity of a certain gene is determined not only by the constitution of the gene system in which it is located but also by the position of the gene within that system. Examples for such position effect are numerous, but Bar is still the clearest demonstration available. As shown by Sturtevant (1925), two Bars, when present in the same chromosome, are more effective than when they are distributed between two chromosomes. A situation similar to Bar was recently described in the case of the dominant Hairy-wing mutant (Demerec and Hoover, 1939). Here also the phenotypic effect of duplication seems to be stronger if the duplicated section is located in one chromosome than when it is divided between two chromosomes. Numerous cases are on record indicating that when chromosomes are broken and reattached in changed positions genes located near breakage points are frequently affected. A recent review of this problem was published by Dobzhansky (1936). Although the possibility of a simultaneous occurrence of a break and a change in a gene adjacent to that break is not excluded, evidence is accumulating (Muller and Prokofyeva, 1935; our unpublished data) which suggests that the phenotypic effect observed in such cases is probably caused by the change in the action of the genes induced by the shift in their position within the gene system.

Interesting evidence of the position effect are various

mottled characters which are readily induced in Drosophila by x-rays. Although the mechanism of this mottling may not yet be satisfactorily explained, it is indisputable that, as first noticed by Schultz (1936), all known mottleds are connected with chromosomal rearrangements which have one break in the proximity of the gene exhibiting mottling and the other break in the heterochromatic region. Thus it seems reasonable to assume that certain genes show mottling phenotype when transferred from their normal position into the heterochromatic region. Recent work of Dubinin (1936) indicates that such position effect may extend an appreciable distance along the chromosome.

Considering all this evidence it seems apparent that the activity of a gene is determined by three internal factors: (1) by the chemical constitution of the gene itself, (2) by the genetic constitution of the gene system in which it acts, and (3) by the position of the gene in the gene system. These three internal factors together with the external factors forming the environment determine the phenotype of the organism. A gene, therefore, should be considered as a unit part of a well-organized system, and a chromosome a higher step in that organization. In that sense genes as individual units with fixed properties do not exist, but their existence as component units of a larger system, with properties partially determined by that system, can not be denied.

In concluding, I will summarize salient points of this discussion. As viewed by at least one geneticist, chromosomes are composed of a fiber-like chromonema which is structurally similar throughout the whole chromosomal complex and to which are attached various radicals. A segment of the chromonema with a number of radicals form a molecular unit which is recognizable through its action as a gene. Indications are that genetically active units contain radicals rich in nucleic acid. The whole chromosomal complex forms a sensitively balanced system. The activity of individual units, genes, is determined by their chemical constitution, by the constitution of the whole system and by their position within that system.

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THE DIMENSIONS AND INTERRELATIONSHIP OF THE RELATIVE GROWTH CONSTANTS

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THE simple power function

$$y = bx^k \qquad (1)$$

has been found to be widely applicable as an empirical representation of the relative growth of two parts (x and y) of an organsim, or of a part (y) and the whole (x). When the equation is applied to the relative growth of the same structures in related types of organisms, characteristic values of the constants b and k are obtained for each type. In certain instances it has been found that these constants are inversely related (Hersh, 1931, 1934; Paulian, 1934; Lumer, 1936). Hersh (1931, 1934) has shown further that the relationship is approximately expressed by a decreasing exponential function of the form

$$b = Be^{-rk} \qquad (2)$$

where B and r are constants.

It was subsequently demonstrated (Lumer, 1936) that if this equation is assumed to be strictly valid, it implies that the relative growth functions in question form a family of curves whose graphs on a double logarithmic grid intersect at a common point with coordinates (r, log B). Since the curves obtained by Hersh were found not to conform to this consequence, it was concluded that equation (2) could be regarded as only a crude empirical approximation.

There remains to be answered, however, the question why an inverse relation between b and k exists. Clearly, the answer must be sought in the nature of the constants themselves. Since the relative growth function is purely an empirical expression, we are unable to attribute to these constants any significance other than that which they

have by virtue of the mathematical character of the equation. It can be shown, however, that regardless of what their theoretical significance may be, the relationship arises simply from the fact that the constant k is dimensionally an intrinsic part of the constant b.

The question of dimensionality in connection with the relative growth function has already been dealt with by Needham (1934), who points out that the two sides of the equation are not dimensionally equivalent. That is, if x and y are masses, then x^k can not in general have the dimensions of mass, and we thus have dimensionally unequivalent terms in the right and left sides of the equation. This means, according to Needham, that the equation "has no true physical meaning, that is to say, no new concept can be deduced from it as it stands, in the sense that the concept of acceleration arises from the relation $f/M = a$ between mass, force and acceleration" (Needham, 1934, p. 82). This point of view, while not entirely incorrect, is somewhat misleading, since it fails to consider the dimensions of the constants, or at least gives the impression that in order to do so, we must know their physical significance.

Now the importance of the principle of dimensional equivalence lies entirely in the requirement that any equation which correctly describes a relationship between measurable quantities must be expressible in a form valid for measurements in any system of units. An equation expressed in such a form is termed *complete*. It can be shown that a complete equation is also dimensionally homogeneous (*i.e.*, all its terms have the same dimensions) provided that it is the only relationship connecting the variables in question (Bridgman, 1931, pp. 36–42). The restriction of completeness, however, is entirely inconsequential, since, as Bridgman (1931, pp. 14–16) shows, any equation which correctly reproduces the results of measurements made with a particular set of units can be made complete by introducing as a factor with each measured quantity a constant of suitable dimensions. In equation

(1), this amounts to assigning suitable dimensions to b. By differentiating (1) we obtain

$$\frac{dy/y}{dx/x} = k,$$

from which it is evident that k, being the ratio of quantities with the same dimensions, is a dimensionless constant, and will therefore not change in value when x and y are measured in different units. On the other hand, b is a dimensional constant which varies with the units of measure employed. Let us suppose that equation (1) has been found to be valid for a particular set of data in which x and y are lengths measured in some particular unit, say millimeters, and that b has been found to have the specific value b_0. We have

$$y = b_0 x^k.$$

To make this equation complete (*i.e.*, valid when x and y are expressed in any other unit of length), we need merely to introduce a factor q, representing the ratio of the new unit to the original one, in the following manner:

$$yq^{-1} = b_0 q^{-k} x^k.$$

This may be written in the form

$$y = b_0 q^{1-k} x^k$$

from which we have

$$b = b_0 q^{1-k} \qquad (3)$$

Thus if the unit of length employed is changed by a factor q, then b varies by the factor q^{1-k}. In other words, b has the dimensions of $1-k$ in length.

All this shows that lack of physical meaning on the part of equation (1), in the sense in which Needham uses the expression, is due not to a lack of dimensional equivalence, but rather to the presence of a complex dimensional constant which we are unable to break down into fundamental components. The present discussion does not contribute anything to our knowledge of the significance of the constants b and k in terms of fundamental concepts; such

knowledge can be gained only through the development of an adequate theoretical basis for the equation. It merely shows that if the relative growth function is to be a complete equation, then, no matter what their theoretical significance may be, these constants must have the dimensions indicated above.

It is particularly to be noted that k is dimensionally contained in b, appearing as a negative exponent. The value of b depends, therefore, not only on the size of the unit in which x and y are measured, but also on the value of k. Moreover, if all other factors remain constant, b will decrease in value as k increases, and we thus have a simple explanation for the existence of an inverse relationship between the values of these constants obtained for related types of organisms. It does not follow that b and k must always be inversely related, for the other factors involved need not remain constant. In particular, situations may arise in which k remains fixed while b varies, or *vice versa*. Examples of the former are not uncommon in practice (*cf.* Sinnott and Kaiser, 1934), but I am unaware of the existence of any examples of the latter.

There is one additional feature of the relative growth function as it applies to ontogenetic processes which enables us to gain some further insight into the composition of these constants. Both x and y are functions of time, and although the time variable does not appear explicitly in equation (1), it is nevertheless present implicitly, since x and y are always measured at the same instant of time. It is thus possible, if the time functions are known, to obtain by eliminating the time variable between them a direct relation between x and y. This has been done for several of the numerous types of empirical equations which have been applied to the absolute growth of organisms or parts of organisms, and it has been found that in most cases the relation thus obtained is, at least as a first approximation, of the form of (1), with the constants b and k expressed in terms of the constants of the absolute growth functions employed (*cf.* Bernstein, 1934; Lumer,

1937; Hamai, 1937; Glaser, 1938). To indicate the general nature of the results, it will be sufficient to consider two examples from Lumer (1937).

A very simple case is that in which each of the parts x and y is an exponential function of time. That is,

$$\begin{cases} y = a_1 e^{r_1 t} \\ x = a_2 e^{r_2 t} \end{cases}. \tag{4}$$

If each of these is solved for t and the solutions are equated, we have

$$y = \frac{a_1}{a_2^{r_1/r_2}} x^{r_1/r_2}.$$

This is obviously of the form of (1), with $k = r_1/r_2$ and $b = a_1/a_2^k$. If x and y are lengths, then it is readily seen that r_1 and r_2 have the dimensions of $(\text{time})^{-1}$, while a_1 and a_2 have the dimensions of length. It follows that k is of zero dimensions, while b has the dimensions of $(\text{length})^{1-k}$.

A second case is that in which x and y are simple autocatalytic functions of time, of the form

$$\begin{cases} \log \dfrac{y}{A_1 - y} = r_1 A_1 (t - t_1) \\ \log \dfrac{x}{A_2 - x} = r_2 A_2 (t - t_2) \end{cases} \tag{5}$$

in which r_1 and r_2 are the velocity constants, A_1 and A_2 the upper asymptotes, and t_1 and t_2 the times at which the points of inflection occur. The elimination of t between these equations yields

$$\frac{y}{A_1 - y} = \frac{1}{C}\left(\frac{x}{A_2 - x}\right)^k$$

where $C = e^{-r_1 A_1 (t_2 - t_1)}$ and $k = r_1 A_1 / r_2 A_2$. If y and x are small compared to A_1 and A_2, this equation is approximated by

$$y = (A_1 / C A_2) x^k$$

Here again k is dimensionless and, since C can be shown to be dimensionless also, b has the same dimensions as in the preceding case.

Thus in these cases, as well as in all the others that have been examined, b can be written in the general form

$$b = PQ_1Q_2^{-k} \qquad (6)$$

where P and k are dimensionless, and Q_1 and Q_2 have the same dimensions as x and y. Now if the quantities PQ_1 and Q_2 are assumed to be constant, equation (6) becomes

$$b = PQ_1e^{-(\log Q_2)k}$$

which is clearly identical in form with (2) if we set $PQ_1 = B$ and $\log Q_2 = r$. On the other hand, if we assume that the relation between b and k is expressed by both (2) and (6), then it follows that

$$PQ_1Q_2^{-k} \equiv Be^{-rk}$$

or

$$\log PQ_1 - k \log Q_2 \equiv \log B - rk$$

and hence that $B = PQ_1$ and $r = \log Q_2$. We have, therefore, a necessary and sufficient condition that (2) hold exactly, namely, that PQ_1 and Q_2 have the same values in all the individuals or groups to which the relative growth function is applied. Where x and y conform to equations (4), this would mean that a_1 and a_2 are fixed, or, in other words, that each of the two parts has the same x- or y-intercept, respectively, in all the cases involved. If the growth of x and y is given by equations (5), then we should have CA_1 and A_2 constant.

Success in fitting equation (2) to a set of values of b and k, however, can not be regarded as a criterion of the constancy of PQ_1 and Q_2. Since k occurs in equation (6) as an exponent, whereas P, Q_1 and Q_2 occur as factors, it is evident that fluctuations in k will affect the value of b to a much greater degree than will equivalent fluctuations in the remaining parameters. Consequently, a fairly good approximation to (2) may be obtained even when PQ_1 and Q_2 vary considerably. Since under these circumstances b is a function of four variables, it is impossible without further information to determine the precise

character of the curve which will be obtained by plotting it against k or against any of the others.

It should be noted that, as can readily be ascertained from (6), b and k need not be functionally related; either may remain fixed while the other varies as a function of the remaining parameters. If, however, the two are related, the relation will tend to be an inverse one except in the unusual circumstance that P, Q_1 and Q_2 exhibit extremely large fluctuations in comparison with those of k.

In view of the foregoing considerations, it seems doubtful whether any really useful results can be achieved by investigating empirically the relation between b and k. These are both complex entities, whose values depend on those of a number of constituent parameters, the nature of which is at the present time extremely obscure. The present analysis shows that this method of attack will not provide any elucidation of these fundamental constants, since first, the existence of the type of relation which has been found to occur in practice is explicable in terms of dimensional considerations without regard to the theoretical significance of the constants, and second, the ability to fit a particular type of equation to experimentally obtained values of b and k does not enable us to draw any conclusions regarding relationships among the constituent parameters.

It should not be inferred, however, that the study of the relation between b and k is devoid of biological interest. With increased knowledge of the time relations of ontogenetic processes, such as embryonic segregations, it may be possible eventually to interpret b and k in terms of fundamental constants denoting specific characteristics of ontogenesis. Under such circumstances, knowledge of their interrelationship may well prove to be of great importance. In the meantime, while it is at least of interest to determine and to compare such relationships in different groups of organisms, this method of approach will not lead directly to an understanding of the fundamental significance of the relative growth constants.

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DIFFERENTIAL GROWTH AND EVOLUTION IN A SUBTERRANEAN ISOPOD

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THE purpose of this paper is to present measurements, analysis and discussion of differential growth and its relation to taxonomy and evolution in a subterranean, freshwater isopod, *Asellus californicus* Miller (1933).

Investigations on the subject of differential growth and its relations to other branches of biology have been greatly stimulated by the publications of Professor Julian S. Huxley, notably his excellent book, "Problems of Relative Growth" (1932), which summarizes the previous work on the subject. Huxley recognizes two types of growth of a part with reference to the body considered as a standard: (1) *heterogonic growth,* in which the growth rate of the part is different from that of the body as a whole, and (2) *isogonic growth,* in which the growth rate of the part and the body are the same. Interstructural heterogony implies changes with age in the relative proportions of parts, a fact of considerable importance in the classification and study of relationships of organisms. There is, however, in practically every instance, a definite relationship between these variables, which may be expressed by Huxley's heterogony formula,

$$y = bx^k$$

where y is the magnitude of the differentially growing structure, x is the magnitude of the body or standard of reference, and b and k are constants. When k equals 1, growth is isogonic, and thus isogony is simply a special case of the law of heterogony. Recently, Glaser (1938) has ably reviewed the quantitative relations of growth, time and form.

Preliminary observations on a series of specimens of

Asellus californicus (Fig. 1) indicated that certain parts, notably the uropods and second antennae, exhibited disproportionate elongation, with growth, in respect to the rest of the body. It was further noticed that, while the basal segment (protopodite) and inner branch (endopodite) of the uropods both showed this differential growth, the exopodite or outer branch of the uropod appeared to grow more in proportion to the rest of the body. As a result, the exopodite appeared "relatively short and almost rudimentary in the larger specimens" (Miller, 1933) compared to the other parts of the uropod. Other structures also seemed to maintain, with growth, their

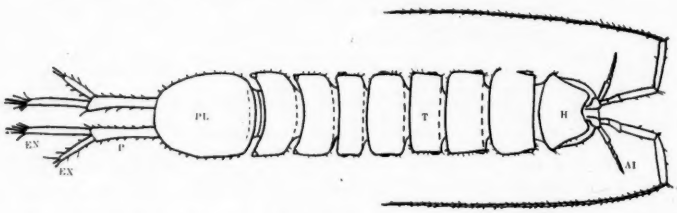

FIG. 1. *Asellus californicus*, dorsal view of male. AI, first antenna; AII, second antenna; H, head; T, third thoracic segment; PL, pleotelson; P, protopodite of uropod; EN, endopodite of uropod; EX, exopodite of uropod.

relative proportions to the body as a whole. Consequently, a series of measurements of various parts of a number of specimens was made and analyzed in order to obtain a better quantitative picture of the growth rates affecting the various parts of the body and their relations to the whole.

The results of these measurements are given in Table I. Measurements were made with a calibrated ocular micrometer and a dissecting binocular.

The first step in the analysis of the data was to determine which of the two measures, body length or body width, was the better standard for comparison of the various structures. Since the coefficient of linear correlation between the two variables was found to be 0.98, it made little difference which we selected. We decided, however,

TABLE I

Asellus californicus. MEASUREMENTS IN MM.

No.	Sex	Body			First antenna	Second antenna	Head length	Thorax length	Pleotelson length	Uropod length	Protopodite length	Endopodite length	Exopodite length
		Length	Width	Index*									
1	?	1.5	.3	4.4	.2	.9	.2	1.1	.2	.2	.09	.09	.09
2	?	1.5	.3	5.0	.2	—	.2	1.1	.3	.2	.04	.1	.09
3	♀	1.9	.4	4.5	.3	1.3	.2	1.3	.4	.3	.1	.2	.1
4	♀	2.3	.4	6.0	.2	—	.3	1.5	.4	.3	.1	.2	.1
5	♂	2.3	.3	6.9	.3	1.1	.3	1.7	.4	.3	.1	.2	.1
6	♀	2.5	.5	5.3	.2	1.6	.4	1.5	.5	.4	.2	.2	.2
7	♂	2.5	.6	4.5	.3	1.7	.3	1.6	.5	.5	.2	.3	.2
8	♀	2.7	.5	5.3	—	—	.4	1.7	.6	.4	.2	.2	.1
9	♀	2.8	.6	4.3	.4	1.9	.3	1.9	.6	.4	.2	.2	.2
10	♀	3.1	.6	4.8	.4	2.1	.3	2.1	.6	.5	.2	.3	.2
11	♂	3.8	.7	5.3	—	—	.5	2.5	.9	.8	.4	.4	.3
12	♂	3.8	.7	5.6	.5	2.1	.5	2.6	.8	.7	.3	.4	.2
13	♀	4.0	.8	5.2	.5	2.6	.5	2.6	.9	.7	.3	.3	.3
14	♀	4.3	.8	5.6	.3	—	.4	3.0	.9	.8	.4	.4	.3
15	♀	4.5	.8	5.5	.6	2.6	.4	3.1	1.0	.7	.3	.3	.3
16	♀	4.5	.9	5.2	.4	—	.6	3.1	.8	.7	.3	.3	.3
17	♂	5.2	1.1	4.9	.7	—	.4	3.7	1.1	1.4	.7	.7	.4
18	♂	6.2	1.3	4.8	—	6.6	1.1	3.4	1.7	2.8	1.5	1.3	.6
19	♂	6.2	1.1	5.6	.9	4.3	.5	4.4	1.3	1.5	.8	.7	.5
20	♂	7.0	1.3	5.5	—	—	.6	5.1	1.4	1.9	1.0	.9	.4
21	♂	7.0	1.3	5.5	1.1	4.3	.5	5.0	1.5	2.8	1.5	1.3	.5
22	♂	7.3	1.3	5.6	1.0	6.0	.6	5.2	1.5	2.3	1.2	1.1	.6
23	♂	7.4	1.3	5.6	.9	6.0	.7	5.3	1.5	2.5	1.3	1.2	.6
24	♂	9.0	1.6	5.7	1.2	8.0	.8	6.4	1.8	4.5	2.3	2.2	.7
25	♂	10.9	1.7	6.4	1.6	11.1	1.1	7.4	2.3	6.8	3.8	3.0	.8
26	♂	11.1	1.7	6.5	1.7	11.9	.9	7.6	2.6	8.3	4.9	3.4	.9

* Index = length/width in ocular micrometer readings.

that body width was more reliable because the measurements of body length were subject to some error due to differential telescoping of the body segments which are rather loosely articulated. Moreover, the head and pleotelson lengths whose growth rates we planned to study constitute roughly one third of the body length.

We next determined by plotting the values for each organ (y) against the corresponding values for body width (x) on various types of graph paper that the best linear trends were obtained in each case on a logarithmic graph (Fig. 2). The formula for these straight lines, of course, is $\log y = \log b + k \log x$, which is simply another way of writing Huxley's heterogony formula. Thus, our data obey Huxley's law of heterogonic growth.

We notice in Fig. 2, however, that there is a marked increase in slope in the lines for each structure (with the possible exception of the head, thorax and exopodite of the uropod) at approximately 1.0 mm body width representing an abrupt acceleration in the differential growth rates beginning at that period in the life of the animal. These changes in growth rates are like those found by Sandon (1937) in the appendages of the crab *Ocypoda*, but are much more pronounced. We shall hereafter refer to the stable period of growth represented by a change in body width from 0.3 to 1.0 mm as the *first stanza*, and that from 1.0 mm to 1.7 mm as the *second stanza* (following Glaser's terminology, 1938).

The next step was to determine the best-fitting lines to the logarithmically plotted data points, *i.e.*, to calculate the constants b and k separately for the two stanzas of growth for each structure. The constants of the best-fitting lines were determined by the method of moments as follows:

$$D = n\Sigma X^2 - (\Sigma X)^2$$

$$b = \log^{-1} \frac{(\Sigma Y \Sigma X^2 - \Sigma X \Sigma XY)}{D}$$

$$k = \frac{n\Sigma XY - \Sigma X \Sigma Y}{D}$$

where n is the number of cases, and X and Y are the logarithms of x and y, respectively (formulae adapted from

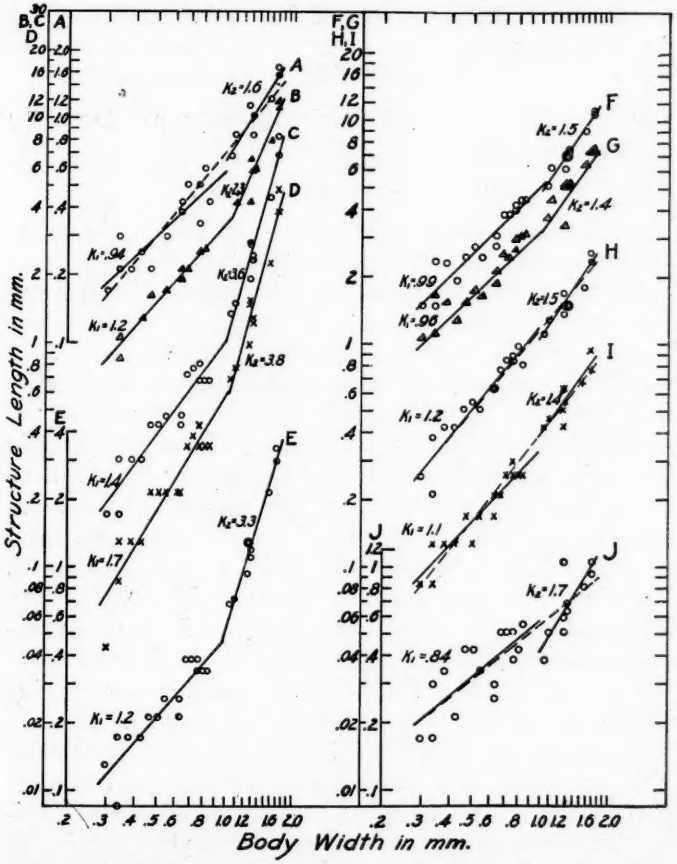

FIG. 2. Logarithmic graphs of structure lengths of (A) first antenna, (B) second antenna, (C) uropod, (D) protopodite of uropod, (E) endopodite of uropod, (F) body, (G) thorax, (H) pleotelson, (I) exopodite of uropod and (J) head plotted as functions of body width in mm. More data near $x = 1$ mm would have closed the gaps between the trend lines of the two stanzas. The dashed lines are the trend lines of the two stanzas combined.

Camp, 1931, p. 106). For those structures whose growth curves did not indicate a large change in growth rates, the constants b and k were calculated for the whole range of body widths, as well as separately for each stanza.

The standard errors of k (SE_k) were determined by the following formula,

$$SE_k = \sqrt{n\, s^2/D}$$

where s is given by the following formula, and the other symbols are the same as defined above,

$$s^2 = [n\Sigma Y^2 - (\Sigma Y)^2 - k^2 D]/n(n-2).$$

These two formulae were adapted from Fisher (1936, pp. 137–148) and Snedecor (1937, p. 113).

We next determined which structures, if any, were heterogonic in growth with respect to body width. Since heterogony exists when k is greater or less than unity, our test for heterogony was simply to determine whether or not k was significantly different from 1, using SE_k for the standard error of the difference, $k-1$. If the critical ratio (t_{k-1}), *i.e.*, the difference $(k-1)$ divided by the standard error of the difference, was 2.5 or more for eight or more measures, the difference was considered to be significant and growth to be heterogonic. For samples of any size, the difference was considered significant and growth heterogonic if P_{k-1} was .05 or less, where P_{k-1} is the probability against the difference being due to chance. (See Table II.—Values of P interpolated from Fisher's Table IV for values of t and "n," where "n" $= n-2$).

As Huxley has shown, the constant b has "no particular biological significance since it merely denotes the value of y when x equals 1 . . . we may call it the fractional coefficient." By a happy coincidence, the marked changes in growth rates indicated by the change of slope, or the intercepts of the lines in Fig. 2, occur in every case at body width of 1.0 mm, *i.e.*, when x equals 1. Hence, the values for b are approximately the same for both stanzas of growth for each organ. In the uropod, for example, b is 1.00 for the range of body width 0.3 to 1.0 mm and exactly the same for the range 1.0 to 1.7 mm body width (Table II). The fractional coefficient, b, of course, is necessary in predicting values of one variable given the other.

The constant k, on the other hand, denotes the constant

TABLE II

SUMMARY OF GROWTH COEFFICIENTS*

$y = bx^k$

Structure (y)	Interval of body width x mm	b	$k \pm SE_k$	Critical ratio t_{k-1}	Probability against heterogony P_{k-1}	Critical ratio $t_{k_2-k_1}$	Probability against $k_2-k_1>0$ P
First antenna	0.3 – 1.0	0.58	0.94 ± .15	0.38	.71		
	1.0 – 1.7	0.66	1.6 ± .25	2.48	.05	1.73	.10
	0.3 – 1.7	0.71	1.2 ± .077	2.76	.013		
Second antenna	0.3 – 1.0	3.41	1.2 ± .085	1.78	.12		
	1.0 – 1.7	3.12	2.3 ± .36	3.76	< .01	3.95	< .01
Body	0.3 – 1.0	5.15	0.99 ± .096	0.077	> .90		
	1.0 – 1.7	4.87	1.5 ± .13	3.70	< .01	1.99	.06
Head	0.3 – 1.0	0.57	0.84 ± .19	0.83	.42		
	1.0 – 1.7	0.42	1.7 ± .45	1.51	.18	1.56	.14
	0.3 – 1.7	0.55	0.81 ± .094	1.97	.06		
Thorax	0.3 – 1.0	3.41	0.96 ± .10	0.45	.66		
	1.0 – 1.7	3.38	1.4 ± .28	1.60	.16	1.66	.11
	0.3 – 1.7	3.71	1.1 ± .052	1.52	.15		
Pleotelson	0.3 – 1.0	1.18	1.2 ± .11	2.13	.05		
	1.0 – 1.7	1.03	1.5 ± .18	2.77	.03	0.99	.33
	0.3 – 1.7	1.14	1.2 ± .048	4.13	< .01		
Uropod	0.3 – 1.0	1.00	1.4 ± .13	2.79	.015		
	1.0 – 1.7	1.00	3.6 ± .32	8.15	< .01	6.07	< .01
Protopodite of uropod	0.3 – 1.0	0.56	1.7 ± .18	3.83	< .01		
	1.0 – 1.7	0.50	3.8 ± .38	7.38	< .01	4.47	< .01
Endopodite of uropod	0.3 – 1.0	0.47	1.2 ± .14	1.09	.30		
	1.0 – 1.7	0.51	3.3 ± .25	9.24	< .01	5.91	< .01
Exopodite of uropod	0.3 – 1.0	0.33	1.1 ± .097	0.58	.57		
	1.0 – 1.7	0.39	1.4 ± .27	1.53	.17	1.23	.23
	0.3 – 1.7	0.38	1.3 ± .054	5.22	< .01		

* b and k were computed from lengths of x and y estimated to one place more than those published in Table I and triply checked from original ocular micrometer readings by different methods of analysis.

differential growth ratio of growth coefficient of the structure (y) relative to the growth of the body or some standard (x) as is evident from the following derivation:

$$\left(\frac{1}{y} \cdot \frac{dy}{dt}\right) = k \left(\frac{1}{x} \cdot \frac{dx}{dt}\right)$$

In words, the relative growth rate of the structure equals k times the relative growth rate of the standard. The biological importance of k resides in the fact that, since k is the exponent in the heterogonic power formula, changes in its value will result in marked visible alterations in the relative proportions of the growing organism, as we shall see later. Graphically represented, k is the slope or tangent of the angle of inclination of the straight lines on the logarithmic graph: the greater the value of k, the steeper the slope.

Considering the values of k in conjunction with their standard errors (Table II), we observe that, during the first stanza of growth, only the uropod and pleotelson are heterogonic in growth. The heterogony of the uropod is due mainly to the marked heterogony of its protopodite (k equals 1.7), since the length of the uropod is the sum of the protopodite and endopodite. The other structures or parts have k values not statistically significantly different from 1.0, and hence are isogonic in growth. During the second stanza of growth, however, there is a simultaneous onset of heterogony in each structure previously isogonic, and a still greater heterogony in the uropod as indicated by the astonishingly large and statistically significant[1] increases in the values of k for this period. In some in-

[1] To test for the statistical significance of these differences between the values of k in the first and second stanzas of growth (k_1 and k_2), we determined the critical ratio (t) by dividing the difference ($k_1 - k_2$) by the standard error of the difference (SE_{1-2}) which is calculated by the following formulae:

$$SE^2_{1-2} = s^2\left(\frac{n_1}{D_1} + \frac{n_2}{D_2}\right)$$

$$\text{where } s^2 = \frac{(n_1\text{-}2)\, s_1^2 + (n_2\text{-}2)\, s_2^2}{n_1 + n_2 - 4}$$

When t is 2.1 or more, for 18 or more measures, the difference is considered

stances, the values of k are doubled during the second stanza of growth. Indeed, the values of k for the uropod and its protopodite and endopodite are extremely high for post-embryonic growth of structures. Other structures which show statistically significant changes in k from the first to the second stanza are the second antenna, and the body (?). The visible consequences of these facts are simply that, during the first stanza of growth, the structures grow more or less in proportion to the body and to each other with the exception of the uropod and pleotelson, which early begin to show relative elongation. During the second stanza of growth, the structures begin to elongate disproportionately with respect to the body. This is especially true in the case of the second antennae and uropods which, in the larger specimens, are extremely elongate relative to the body.

The uropods show in striking fashion the consequences of changing and different values of k in its constituent parts. Of the parts of the uropod, the protopodite alone is significantly heterogonic in the first stanza, but in the second stanza the endopodite speeds up its relative growth rate and contributes almost equally with the protopodite to the extreme heterogony of the uropod. As a matter of fact, the difference between k for the protopodite and endopodite in the second stanza is not significant, and hence these two structures constitute, with respect to each other, an isogonic diad. In contrast, the exopodite is isogonic in the first stanza, and only moderately heterogonic in the second stanza. (Although P_{k-1} for the exopodite in the second stanza indicates 17 chances out of 100 against its k of 1.4 being different from 1.0, we can still guarantee heterogony in the second stanza, since the k of 1.3 for the whole range or both stanzas together proves to be significantly heterogonic.) As a consequence of these changing

significant; or better, when the corresponding P values (probabilities against significance) are less than .05. "n" in Fisher's table, in this case, is $(n_1-2)+(n_2-2)$. The same formulae were also used in determining significance of differences between any two k values in Table II, regardless of stanza or structure.

and different values of k, the proportionate lengths of protopodite, endopodite and exopodite change roughly from 4:6:5, respectively, in the smallest specimens to about 6:4:1 in the largest (Fig. 3).

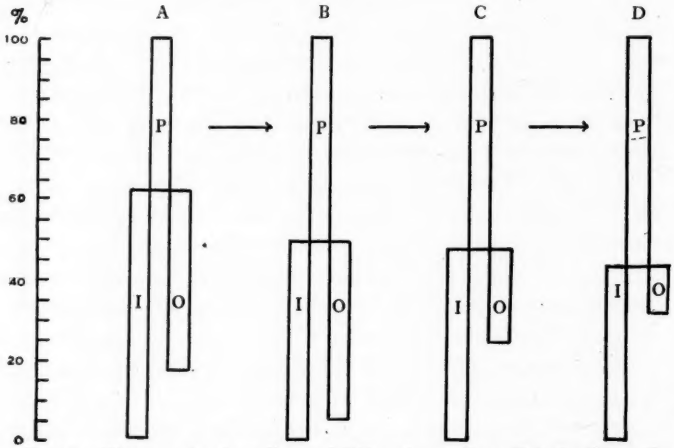

FIG. 3. Diagram showing effects of different growth coefficients (k) on the relative proportions of parts of the uropod in *Asellus californicus*. *P*, protopodite; *I*, inner branch (endopodite); *O*, outer branch (exopodite); *A*, *B*, *C* and *D*, average percentage proportions of the uropods of specimens Nos. 1–3, 13–15, 18–20, 24–26, respectively.

The differences in growth between the first and second antennae are slight, not significant ($t = .99$) in the first stanza, but are fairly marked in the second stanza. Prior to our observations, there must have been differences in the growth of these two appendages or in the time at which growth started since, in the smallest specimens measured, the second antennae were already four times longer than the first antennae, (Table I, Nos. 1 and 3). During the first stanza the ratio between the lengths of the two antennae increases but slightly, if at all. With the advent of the greater heterogony exhibited by the second antennae in the second stanza, however, the ratio between the lengths of the two antennae becomes progressively greater until in the largest specimen measured (Table I, No. 26), the second antenna is seven times the length of

the first. That it should be the second antenna instead of the first which suffers the extreme elongation is not surprising since, in the order Isopoda, the first antennae are always of lesser size and importance (except in the Tanaioidea), and in the land isopods (Oniscoidea) the first antennae are rudimentary vestiges. With respect to the body, the second antennae change from six tenths the body length in the smallest specimen to equal the body length in the largest specimens.

The head is rather variable in length measures, especially in the first stanza, as indicated by the scattering of the data points (Fig. 2) and the large standard errors of k (Table II). The growth tendency in the first stanza is toward negative heterogony, since k is less than 1, but this value is definitely not significantly different from 1. Growth changes in the second stanza to positive heterogony, but again we can not statistically guarantee heterogony, or that the change from stanza one to stanza two is significant.

The growth rate in length of the body is conditioned by the growth rates of its constituent parts with the head and pleotelson together contributing about one third, and the thorax the other two thirds. In the first stanza, the tendency toward negative heterogony in the head is balanced by the positive heterogony in the pleotelson, and, since k for the thorax is 1.0, we should expect k for the body length to be about 1.0, which indeed it is. In the second stanza, the positive heterogony of the head, abdomen and possibly the thorax make the body as a whole heterogonic in length. The differences of k between the head, thorax and abdomen are not statistically significant in the second stanza, and hence these structures, although heterogonic with respect to body width, constitute an isogonic triad.

The general pattern of growth in the long axis, in spite of differential heterogony, is orderly, as illustrated by the simple growth gradients in Fig. 4. The protopodite of the uropod is a center of differential growth intensity in both stanzas, extremely high in the second stanza with the endopodite as a close rival. In the second stanza, the second

antenna is also a center of differential growth, and, from these two centers in the opposite terminal appendages of the body, the gradient of "growth potential" slopes proximally down to the middle of the body. We might crudely picture the animal in the second stanza as being stretched out by its ends with the central part of the body relatively inelastic. It is of some interest that the exopodite which is articulated to the protopodite at a considerable angle

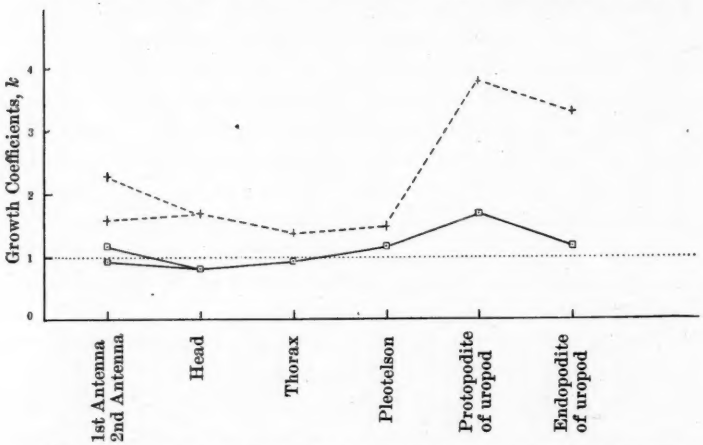

FIG. 4. Growth gradients in the longitudinal axis, *Asellus californicus*. Solid line, first stanza; dash line, second stanza; dotted line, line of isogony.

to the long axis of the body (see Fig. 1) is practically isogonic throughout, whereas the protopodite and endopodite which parallel the long axis of the body are strongly heterogonic.

The most plausible explanation for the existence of stabilized disproportionate growth is that the growth rates of each structure is determined wholly, or in part, by different rate genes. The synchronous onset of heterogony and the existence of orderly patterns of growth, *i.e.*, the simple growth gradients, indicates the probability that there are also genetic factors controlling growth of the organism as a whole, which express themselves at a certain period late in the life of the animal. Through the work of Goldschmidt and other physiological geneticists

we are learning more and more of the existence and behavior of genes determining rates of processes and of the timed action of genes.

The possibility that the heterogony of the second stanza may be a male secondary sexual or sex-limited character is strongly indicated by the fact that all the specimens in the second stanza of growth are males (Table I). This may be due to the small size of the sample or it may indicate a real sexual difference. In the latter case, the onset of heterogony may be coincident with or conditioned by the sexual maturity of the male. Unfortunately, we do not know when sexual maturity occurs in either sex. None of the females was carrying young in the brood pouch, and the small males show apparently as complete development of the second pleopod (copulatory organ in the Asellidae) as the larger ones.

The possibility that the extreme heterogony of the second antennae and uropods may be due, at least in part, to a functional hypertrophy in compensation for loss of eyes can not be excluded, since both of these structures undoubtedly subserve a tactile as well as other sensory functions. That the heterogony of these organs is not entirely due to functional hypertrophy, however, is evident from the fact that the disproportionate elongation is not gradual but increases sharply as we have seen when the animal reaches a certain size. But the functional necessity of sensory contact with the environment is presumably of equal intensity, at least throughout the span of life here studied, and hence there is no apparent functional explanation of the sharp increase characterizing the second stanza of growth. Rather, the synchronous onset of heterogony and the fact that non-adaptive structures are also involved suggests intrinsic factors.

The elongation of the body, especially of the ends of the body and their terminal appendages, is of special evolutionary significance because this feature is one of the striking ways in which subterranean Crustacea differ from the surface species. All the subterranean Asellidae are like our *Asellus californicus* in that they are more elongate

with longer heads, pleotelsons, antennae and uropods than their surface relatives. They also resemble each other in absence of eyes (or rudimentary eyes) and loss of pigmentation resulting in albinism. Elongation, at least of the tactile organs, and the other features mentioned are more or less obviously adaptations to the similar and unique conditions of existence characteristic of subterranean environments the world over (stygian darkness, uniform cold temperatures, etc.). The superficial resemblance of the subterranean asellids to each other resulting from like adaptation to similar environment camouflages their real relationships to different surface species in the environs of their habitats, and obscures the fact that the underground species have arisen independently and at different times in various parts of the world. We have here a clear case of convergence in evolution.

This brings us face to face with the chaotic status of the classification of the subterranean asellids. To review the situation briefly, the subterranean asellid species of the United States and Japan, undoubtedly polyphyletic in origin, are artifically grouped together under the generic name *Caecidotea* Packard, 1871, which is poorly distinguished from the genus *Asellus,* the chief differences being the above-mentioned convergent characters. The subterranean asellids of the rest of the world, possessing the same convergent characters, are properly recognized and classified as belonging to diverse lines of *Asellus. Caecidotea* has been declared invalid by various authors (for review see Miller, 1933) on the grounds that its species have arisen independently and at different times from various species of *Asellus,* that its species are more closely related to neighboring surface species than to each other, that its characters are convergent characters not indicative of true relationships, and that even these characters are not good diagnostic or distinctive features, since they intergrade with surface species. If further reasons for invalidating *Caecidotea* were necessary, we might add that, judging from our findings in *Asellus californicus* (which possesses the characters ascribed to *Caecidotea*),

the relative elongation of the body, its parts and appendages, which is one of the main distinguishing features of *Caecidotea,* is not a constant character, but it changes with age in individuals of the same species due to heterogonic growth.

Even in making distinctions between species, our studies have shown that relative length proportions of parts are not reliable characters in this group since heterogony may be at work. But we could give as distinctive taxonomic characters for heterogonic parts "their growth constants. . . . and the absolute size at which heterogony (if not continuous or uniform) begins" (Huxley). We do not anticipate that this refinement will be generally employed, however, because of the difficulty of ascertaining these values as compared to the ease in finding the usual and more obvious qualitative characters. Furthermore, a sample of about twenty-five specimens representative of the growing period is necessary to give us accurate values of the growth constants, but such a sample is comparatively rare in our collecting experience. Many are the species described from a single specimen or a fragment, and a great many more from just a few specimens. We do caution against the common, bald use of relative proportions of parts as important specific characters when heterogony is suspected.

Our final interest in heterogonic elongation is in attempting to determine how it might have originated in *Asellus californicus* and other subterranean forms. If our assumption is correct that the rate of growth is gene-determined, we might further assume that parallel mutations in these rate genes in the various ancestral species resulted in the positive heterogonic elongation of the subterranean species. Assuming the onset of heterogony observed in *A. californicus* to be the expression of the mutant rate genes, the mutation expresses itself relatively late (i.e., at the beginning of the second stanza) in development, as is often the case with recent mutations.

Whatever the cause of heterogony, the result is a recapitulation in that the young subterraneans are more like

the present-day adult surface species and supposed ancestral species in body proportions than are the older specimens. The uropods in *Asellus californicus* illustrate this recapitulation very nicely. In the younger specimens, the basal segment of the uropod is relatively short, its two branches subequal in length (Table I, Nos. 1 and 2), which is the primitive condition of this biramous appendage and like the uropods in the adults of the majority of surface species of *Asellus*. As the animals grow older, the uropods diverge more and more from the ancestral or surface species type, especially with the onset of unequal heterogonic growth in its parts. Judging from the figures of uropods in other subterranean species which show elongated protopodites and endopodites and relatively short exopodites (Van Name, 1936), and from remarks by various authors concerning variation with age in the comparative lengths of these parts, we suspect the same phenomenon of recapitulation due to heterogony could be demonstrated in a series of specimens in other subterranean isopods.

A comparative study of the growth rates in subterranean species with those in related surface species would undoubtedly provide us with further information bearing on the problems of the origin and evolution of subterranean Crustacea.

SUMMARY

(1) Measurements were made of body length, width and the lengths of antennae, head, thorax, pleotelson and parts of the uropod of twenty-six specimens of *Asellus californicus* ranging in size from 1.5 by 0.3 mm to 11.1 by 1.7 mm in order to obtain a quantitative picture of differential growth in this subterranean isopod.

(2) The data obtained were analyzed and found to conform with Huxley's law of heterogonic growth, using body width as the standard of comparison.

(3) The differential growth ratio (k) for each structure was not uniform over the entire range of observation but increased abruptly at the middle of the range, dividing the period of growth studied into two uniform stanzas.

The constants in the heterogony formula were calculated separately for each structure for the two stanzas, as well as for the entire range when the change from first to second stanza was slight or not statistically significant.

(4) Within the range of body width 0.3 to 1.0 mm, growth in length is practically isogonic for all structures except the pleotelson and the uropod, which is heterogonic, due to the marked heterogony in its basal segment.

(5) In the second stanza (body width of 1.0 to 1.7 mm) there is a simultaneous and significant onset of heterogony in all structures, with the possible exception of the thorax, although in some cases the increase in the value of k from first to second stanza is not significant. Extreme heterogony is exhibited by the protopodite and endopodite of the uropod with k values of 3.8 and 3.3, respectively.

(6) Heterogony is not exhibited to the same degree in all structures or parts and the visible consequences of differences in the growth coefficient, k, are described in terms of changing proportions of parts with growth. The best example is the uropod, in which, as a result of different degrees of heterogony in the protopodite and endopodite and the practically isogonic growth of the exopodite, the proportionate lengths of protopodite, endopodite and exopodite change from about 4:6:5, respectively, in the smallest specimens to about 6:4:1 in the largest.

(7) The growth gradient in the longitudinal axis of the body shows, in the first stanza, a center of differential growth in the protopodite of the uropod from which the gradient slopes down proximally to the head and distally to the endopodite. In the second stanza, the growth gradient features two terminal centers of high differential growth, the second antenna and again the protopodite which is now an extremely high center. From these two distal centers, the growth gradient slopes down sharply to the middle of the body. The whole gradient in the second stanza is significantly elevated above the level line of isogonic growth.

(8) Rate genes affecting the various parts of the body and possibly the body as a whole are suggested as the

cause of disproportionate but orderly growth. That the heterogony of the second stanza may be a male secondary sexual or sex-limited character is a strong possibility. Reasons are given to show that functional hypertrophy is an inadequate explanation for the extreme heterogony of the second antennae and uropods.

(9) The taxonomic and evolutionary significance of heterogonic elongation is discussed. Relative elongation is shown to have no systematic value in separating the subterranean isopods as a genus (*Caecidotea*) from the surface forms not only because it is a convergent character but also because it is not even a constant specific character, since relative elongation varies with age in individuals of a species as a result of heterogony. Other reasons for invalidating this unnatural genus are reviewed. The elongation distinguishing subterranean forms from surface species possibly resulted in consequence of parallel mutations of rate genes in the ancestral surface species. If so, the mutant genes express themselves relatively late in our *Asellus californicus*. The heterogonic elongation of parts in this subterranean isopod results in a recapitulation of ancestral body proportions.

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ON THE MEASUREMENT AND INHERITANCE OF SEXUAL MATURITY IN TURKEYS (MELEAGRIS GALLOPAVO)

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USING age at first egg as the measure of sexual maturity, Hays (1924) found that early sexual maturity in the domestic fowl differed from late sexual maturity by a dominant sex-linked gene E and a dominant autosomal gene E^1. Warren (1934) confirmed these results but did not consider it definitely established that there was only one pair each of autosomal and of sex-linked genes involved.

Sexual maturity in the domestic fowl is, according to Lerner and Taylor (1937), best measured by the age at first egg, while in turkeys date of first egg is more closely correlated with egg production than is age at first egg (Asmundson, 1938). This suggests that in the case of turkeys, date of first egg is a more useful measure of sexual maturity than age at first egg. In order to obtain further evidence on this point, the trapnest records available for pedigreed turkeys hatched in 1931 to 1937 were analyzed. This paper presents the results of that analysis, together with data on birds selected for early and late maturity and on the progeny obtained from reciprocal crosses of birds in the early and late maturing groups.

MEASURING SEXUAL MATURITY

The stock used for the purpose of determining the best method of measuring sexual maturity consisted mostly of pedigreed Bronze turkeys. A few birds belonging to other varieties and crosses between these were included. The proportion of crossbreeds was small; a considerable part of the Bronze hens kept were inbred. The birds were hatched in the years 1931 to 1937 and, with five exceptions, started to lay the year following the year of hatch. These five birds were hatched in 1937 and started to lay late in December of the same year.

Table 1 shows the number of birds available each year. The date of the first hatch is given in days from the first

TABLE 1

INFLUENCE OF TIME OF HATCH ON AGE AND DATE OF FIRST EGG

Year	1932	1933	1934	1935	1936	1937	1938
Total number of birds	61	67	91	74	73	116	116
Date of first hatch	103	103	108	86	93	92	96
AGE AT FIRST EGG IN DAYS							
Average age, hatch 1	318.6	338.6	311.2	332.4	326.8	335.3	289.9
Decrease in average age:							
Hatch 1–2	−11.1	−15.1	−9.4	−18.2	−14.8	−18.2	+5.3
Hatch 2–3	−14.4	−17.5	−14.5	−6.1	−15.4	−4.1	−3.7
Hatch 3–4	−12.6	−5.4	−9.8	−11.1	−9.2	−17.2	−10.6
Hatch 4–5	−9.8	−11.5		−16.6		−10.3	
Hatch 5–6	−2.4						
DATE OF FIRST EGG IN DAYS							
Average date, hatch 1	55.7	75.6	54.2	53.4	54.8	61.3	29.9
Change in average date:							
Hatch 1–2	+2.1	−2.1	+4.6	−3.7	−1.8	−4.2	+19.3
Hatch 2–3	+1.1	−2.5	−0.5	+7.4	−1.4	+8.9	+10.3
Hatch 3–4	+3.1	+8.6	+4.2	+2.9	+4.8	−3.2	+ 3.4
Hatch 4–5	+3.7	+2.5		−3.6		+3.7	
Hatch 5–6	+9.0						

of the year. Thus birds hatched at 103 days were hatched on April 13. Hatches were spaced two weeks apart; hence the last hatch was six to ten weeks later than the first hatch.

Age at first egg is definitely influenced by the date when the birds were hatched. Except in 1938 the age at first egg of the birds in the second hatch decreased on the average by 9.4 to 18.2 days below the average age of the birds in the first hatch. The birds in the last year (1938) were exceptional in that the birds in the first hatch matured at an earlier average age than those in the second hatch. This was due to the fact that the birds out of the early- and late-maturing strains discussed below were not distributed at random. Most of the birds from early-maturing strains were in the first two hatches, while the later-maturing strains were represented in the second and later hatches. Except for 1938 there was a fairly consistent decrease in total average age from the first to the fourth hatch of 33.7 to 39.5 days or an average of 11.2 to 13.2 days for each 14 days' delay in hatching. These figures are calculated from those shown in Table 1. Age at first

egg does not, however, decrease indefinitely with delay in hatching. This is indicated by the results for 1932, where the decrease in age from the fifth to the sixth hatch was only 2.4 days with a corresponding increase in date of first egg.

Date of first egg (in days from the first of the year in which the record was made) is not consistently influenced by date of hatch early in the season. This is particularly true of the first three hatches or up to about the first week in May. After that there is a slight delay in date of first egg, in most cases, with a decided increase after the middle of June for the one year in which data are available.

Correlation coefficients between the date of hatch and age at first egg and date at first egg were computed. Only the data for the years 1933 to 1937 inclusive will be considered here, since the birds were distributed at random through not more than five hatches in these years. The coefficients of correlation for date of hatch and age at first egg were consistently negative ($r = -.561$ to $-.911$), while there was no consistent significant correlation between date of hatch and date of first egg ($r = .028$ to $.466$). The regression of age at first egg on date of hatch varied from $y = -.8018x$ to $y = -1.0556x$ in the five years 1933 to 1937. The coefficients of correlation confirm the results presented in Table 1, that age at first egg is consistently influenced by date of hatch but that date of first egg is not.

Inherited Differences in Sexual Maturity

The data presented on the two measures of sexual maturity considered indicate that date of first egg is a better measure of sexual maturity than the actual age at first egg. Table 2 presents an analysis of variance of male families for date of first egg and age at first egg in the years 1933 to 1938, inclusive. Only families comprising three or more full sisters were used. It will be observed that no inherited difference could be demonstrated for age at first egg in the first three years, while in the last three years

TABLE 2

ANALYSIS OF VARIANCE BASED ON FULL SISTER FAMILIES OF THREE OR MORE BIRDS

Year	Age at first egg					Date of first egg. The degrees of freedom are the same as given under age at first egg		
	Between means of sires		Within means of sires		F	Between means of sires	Within means of sires	F
	Degrees of freedom	Mean square	Degrees of freedom	Mean square		Mean square	Mean square	
1933	4	468.0	62	239.8	2.03*	120.7	45.7	2.64‡
1934	5	614.0	77	345.2	1.78*	1209.0	162.4	7.45†
1935	7	928.2	58	791.6	1.17*	1164.4	169.0	6.78†
1936	6	1672.3	48	258.0	6.48†	1008.1	166.5	6.05†
1937	6	938.3	62	168.8	5.56†	550.0	57.3	9.60†
1938	7	3625.1	78	394.8	9.18†	5304.5	162.5	32.64†

* These values are below the 5 per cent. point for P.
‡ The value of the 5 per cent. point for P is 2.52.
† These values exceed those for the 1 per cent. points for P.

such inherited differences existed. Apparently variance within families became less as a result of inbreeding, while selection increased the variance between families. While the value of F (see Snedecor, 1934) for 1933 is low, it is evident that there were genetic differences in sexual maturity as measured by date of first egg in all the years. Data for age at first egg, when corrected for date of hatch, gave similar results to date of first egg but appeared to have no advantages over the latter as a measure of sexual maturity in the turkey. Date of first egg has, however, definite advantages over age at first egg, since it is little influenced by date of hatch within the average hatching season and can moreover be used to demonstrate genetic differences in sexual maturity in populations that are hatched at random and are so heterozygous that age at first egg can not be used.

Selections were started for early and late maturity in 1933. Several families of the bronze variety were selected and were inbred in an attempt to obtain reasonably homozygous stock. Brother to sister and son to dam matings were made. One line selected for early maturity and one line selected for late maturity gave reasonably good fertility and hatchability, while most of the others had to

be eliminated because of their failure to reproduce themselves satisfactorily. The basis of selection was date of first egg. Complete families were kept, but the number of individuals available was usually small (Table 3.) The

TABLE 3

MEAN DATE OF FIRST EGG IN DAYS FROM THE FIRST OF THE YEAR IN WHICH THE RECORD WAS MADE, TOGETHER WITH THE STANDARD ERROR OF THE MEAN

Year	Early maturing strain		Late maturing strain		*Early* male × *late* females		*Late* male × *early* females	
	No. of daughters	Mean date	No. of daughters	Mean date	No. of daughters	Mean date	No. of daughters	Mean date
1934	9	42.44 ± 4.53	7	72.71 ± 1.43				
1935	14	45.86 ± 3.74	7	79.29 ± 2.99				
1936	14	46.00 ± 3.32	11	70.36 ± 1.63				
1937	22	58.86 ± 1.61	7	73.57 ± 1.18	8	58.88 ± 3.18	13	69.23 ± 1.14
1937			6	78.17 ± 1.85				
1938	20	44.25 ± 5.06	10	80.10 ± 1.96	11	61.73 ± 2.44	7	69.71 ± 2.19
1938	13	14.85 ± 3.45	5	85.80 ± 2.16				

data in Table 3 are arranged so that each group in any one line is the progeny of a single male. Thus the birds trapnested in 1934, 1935 and 1936 were in each year out of one early male and one late male. The same early male that sired all the early-maturing females kept in 1937 was crossed onto the late-maturing females, while the late-maturing male in that line was mated to the early females. It will be observed that the early male that gave the earliest-maturing progeny was not mated to late-maturing females, and hence no F_1 progeny were available from him.

The differences between the early- and late-maturing lines were all highly significant, although the mean date of first egg varied considerably from year to year. In 1937 and 1938 a few female progeny were available from each of two different males of the late-maturing line. There were slight differences between the progeny of these males in each case, but these differences were just under the statistically significant value in 1938 and barely significant in 1937. In 1938 the progeny of two males in the early-maturing line were available. These two male families undoubtedly differed genetically, one being considerably earlier maturing than the other. This shows

that the early-maturing line was still heterozygous after four generations of selection.

Reciprocal crosses between the early- and late-maturing lines were made in 1937 and 1938. The results differed according to the way the cross was made. When an early-maturing male was mated to late-maturing females, the progeny were significantly earlier maturing than when the reciprocal cross was made by mating a late-maturing male to early-maturing females. In 1937 the progeny of the early male x late females matured at the same time as the birds in the early-maturing line; whereas in 1938 the birds out of this cross were later maturing than the early-maturing line. The birds out of the reciprocal cross were earlier maturing than the birds in the late-maturing line. These results, therefore, clearly indicate that there are both sex-linked and autosomal genes involved.

Discussion

The fact that both sex-linked and autosomal genes apparently control sexual maturity in turkeys indicates that the hereditary mechanism is similar to that in the domestic fowl (Hays, 1924; Warren, 1934). Perhaps they have a common hereditary mechanism governing sexual maturity which has not been changed fundamentally during the many thousands of years that these species must have been undergoing evolutionary changes separately. In any case, both domesticated turkeys and chickens comprise a mixture of genotypes which represent different combinations of sex-linked and autosomal genes determining sexual maturity.

There is some evidence that more than one pair of sex-linked and of autosomal genes are involved in determining sexual maturity in turkeys. This is indicated by (1) the differences between the date of first egg of the birds out of the matings of the early-maturing male with late-maturing females in 1938 as compared with that of the birds in the early-maturing line, (2) the differences between the progenies of the early-maturing males in 1938,

(3) the apparent differences in the maturity of the daughters of the late-maturing males in 1937 and 1938. More evidence is needed before definite conclusions can be drawn.

The data presented in this paper and elsewhere show that date of first egg is the better measure of sexual maturity in turkeys, while in the case of chickens, age at first egg is the better measure of sexual maturity (Lerner and Taylor, 1937). Chickens usually start to lay at five to seven months of age. Waters (1937) found that chickens reach maximum body weight at about ten months of age or approximately three to five months after they lay their first egg. Unpublished data from the California Agricultural Experiment Station show that turkey hens reach their maximum first year weight at about forty-four weeks or approximately eleven months. This maximum first year weight is reached by both species in the spring, after which there is a decrease in weight during the summer months followed by an increase in weight during the next autumn. In the case of turkeys the age at first egg will usually vary from about eight months to over eleven months with an average of approximately ten months. The turkey therefore starts to lay considerably later than the chicken and at a time just before it has reached the initial or first year peak in weight. The turkey is thus usually physically mature, in the sense that it is nearly full grown, when it starts to lay, whereas the chicken is relatively immature and continues to grow for some time after it starts to lay.

These differences in the average age of the turkey and chicken may be stated in a different way, namely, that the domesticated chicken tends to start laying at a certain age, while turkeys tend to start laying at a certain season of the year. From this it may be inferred that environmental factors play a much more important role in determining the beginning of egg production in turkeys than in chickens. The most important difference between the two species, however, appears to be a difference in the thresh-

old of response to environmental stimuli. In the chicken this threshold appears to be so low that the female starts to lay at an early age. Physical maturity appears to be an important, if not the determining, factor. The turkey, on the other hand, appears to have a high threshold of response. It is sufficiently mature physically to lay long before it usually does. That this is so is indicated by the results of Scott and Payne (1937), and others, who were able, by the use of artificial light, to induce turkeys to lay about two months earlier than birds kept under ordinary conditions. Their results agree with those reported by Rowan (1925) for juncos, and Bissonnette (1930) for starlings and indicate that light, if it is not the only factor, is at least the most important one in the environment which influences sexual maturity (see also Rowan, 1938). Bissonnette and Csech (1938) have also shown that the long periods of daylight to which winter-hatched pheasants are subjected in midsummer will induce them to start laying at an unusually early age. This finding and the observation that turkeys raised in southern California sometimes start to lay in the autumn when less than seven months of age, while this rarely occurs in the northern part of the state, is further proof that at least some species of birds can lay much earlier than they ordinarily do and that they are usually prevented from laying at an early age by an unfavorable environment.

Since light is the most important environmental stimulus to sexual maturity, it may be supposed that the genotype determines the threshold of response to that stimulus. The genotype also presumably determines the age at which the bird becomes sufficiently physically mature to respond to light. If the assumption that the main difference between early and late-maturing strains of turkeys and between turkeys and chickens is a difference in their threshold of response to light, it is tempting to infer that light would not appreciably lower the age at sexual maturity of early-maturing strains of chickens, while in the case of most strains of turkeys artificial light should induce earlier sex-

ual maturity by providing a stimulus well above the threshold required to start the chain of events that lead up to egg production. Nevertheless, in view of the fact that Curtis (1914) has recorded the case of a Barred Plymouth Rock which started to lay when three months old, it would be extremely hazardous to assume that even early-maturing strains of chickens can not be induced to start laying earlier than they usually do by providing the proper stimulus. There is actually no acceptable experimental evidence to show that domesticated chickens will not mature sexually earlier (and start laying earlier) if subjected to artificial light and hence no experimental basis for the assertion that they differ in this regard from wild birds (Rowan, 1938) and from turkeys. The weight of chickens and turkeys at the time they start to lay when compared with their maximum first-year weight does, however, indicate that physical maturity is ordinarily a more important factor in determining the onset of egg production in chickens than in the case of turkeys.

Summary

Statistical analysis of the trapnest records of turkeys extending over seven years shows that date of first egg is a better measure of sexual maturity in turkeys than age at first egg. Date of first egg is little influenced by variation in the time of hatch within the normal hatching season of almost two months, whereas age at first egg is influenced, the later-hatched birds starting to lay when younger than the earlier-hatched birds. Moreover, genetic differences in sexual maturity can more readily be demonstrated with date of first egg than with age at first egg.

Selection for early and late sexual maturity was effective in establishing lines that differed significantly in date of first egg. When these lines were crossed the results differed according to which way the cross was made. The progeny of *late* male x *early* female laid earlier than the birds in the late-maturing line but later than the progeny

of the reciprocal cross. In at least one case, the progeny of the latter (*early* male x *late* female) laid just as early as the birds in the early-maturing line. It is concluded from this that sexual maturity in turkeys is determined by both sex-linked and autosomal genes.

The body weight of turkeys at the time they lay their first egg is usually near the maximum first-year weight, whereas chickens start to lay three to five months before they reach maximum first-year body weight. It is suggested that physical maturity is a less important factor in determining the onset of egg production in turkeys than in chickens and that turkeys have a higher threshold of response to environmental stimuli than do chickens.

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SHORTER ARTICLES AND DISCUSSION

PRECIPITIN ABSORPTION WITH *DROSOPHILA* ANTIGENS

Introduction

The object of this report is to show the value of precipitin absorption in defining antigenic relations among *Drosophila* species. Many investigators have performed absorption experiments wherein use was made of agglutinating particles such as blood corpuscles or bacteria. Only a limited amount of work has been carried out in which precipitins have been absorbed. Hektoen and Delves (1932), Duncan (1932), Stone and Garrod (1931) and Lamb and Lamb (1935) have found various technics of precipitin absorption useful in determining antigenic relations of various species. In connection with *Drosophila,* Russian investigators have reported that by the absorption of complement fixing antibodies, they have been able to detect immunologically the presence of the Y-chromosome in the XY and XXY individuals of *D. melanogaster* (Levit, Ginsburg, Kalinin and Feinberg, 1936).

Duncan's work (1932) is of particular interest to us here, because he applied the optimal proportion technic, suggested by Dean and Webb (1926), to the precipitin absorption technic. In this way he was able to determine the exact quantities of antigen and antibody necessary for maximum absorption, without the interference of either serum or antigen excess. The present work has been preceded by a series of tests in which the optimal antigen-antibody ratios were determined. In so far as it was possible, the data obtained in the prior work were used in estimating the relative quantities of antigen and antibody necessary for complete absorption.

Materials and Methods

Antisera were prepared in the manner indicated in earlier reports; and antigens were prepared by extracting with saline the ether-insoluble fractions of the flies (Cumley, in press). All antigens were adjusted for equivalent nitrogen contents, as determined by the micro-Kjeldahl test. Antisera and antigens had been passed through Seitz filters, were preserved with Merthiolate Solution, and were kept at ice-box temperatures. Aseptic

precautions were taken in handling reagents, and no growth appeared in any of them during the course of these experiments.

In absorbing an antiserum with an antigen, 2 cc of the desired antiserum dilution were placed with the same quantity of properly diluted antigen. The mixture was placed in the ice-box for 24 hours, after which it was removed and centrifuged at high speed for 7 minutes. The supernatant liquid was pipetted off and tested by the appropriate reagents. All absorptions and subsequent tests were carried out in Wassermann tubes. Although in these experiments time was kept, as is usual for optimal ratio work, the author found that the determination of the optimal ratio in the supernatant fluids, after absorption, was virtually impossible. Duncan reported similar difficulty.

The antisera to *D. caribbea* and *D. virilis* were absorbed with the antigens of *D. caribbea, D. virilis, D. mulleri* and *D. melanogaster.* Absorption tests could not be performed on additional antisera because of insufficient quantities of reagents. In fact, some of the necessary cross tests could not be made with the above reagents, because of lack of materials. Using quantities previously determined by the optimal ratio work, several proportions of antigen and antiserum were made up, the object having been to carry out absorption at or near the optimal ratio of flocculation. Also, mixtures were made in which the presence of an antigen or antibody excess was known or suspected. Table 1 is a presentation of the antigen-antibody mixtures that were used in the

TABLE 1

Antigen dilutions	*D. caribbea* antiserum dilutions			*D. virilis* antiserum dilutions
	1:1	1:2	1:4	1:1
D. caribbea 1:20			×	
1:40			×	×
1:60				×
1:80			×	
D. melanogaster 1:20		×	×	
1:30		×	×	
1:40				
D. mulleri 1:15	×			
1:20	×			×
1:30				×
1:40				×
D. virilis 1:15	×			×
1:20	×			×

absorptions. An "×" denotes the ratio of antigen to antibody in any given mixture. For example, 2 cc of *D. caribbea* antiserum, diluted 1:4, was mixed with 2 cc of *D. caribbea* antigen, diluted 1:20.

Flocculation tests were run on *D. caribbea* antiserum and *D. virilis* antiserum, against their homologous antigens, to determine the end-points of their reactions. The antisera were then divided into separate portions, and each portion was absorbed by a different antigen. As indicated in Table 1, each species antigen was mixed with a given antiserum in ratios which were thought to include and usually extend to each side of the optimal ratio of precipitation. After absorption, each mixture was centrifuged and the supernatant liquid collected. This fluid was then tested against the homologous antigen, in order to determine relatively how much material had been removed by precipitation during the absorption process. The supernatant liquid was also tested against the absorbing antigen, in order to determine whether all the antibody in common with the antigen had been absorbed at that particular antigen-antibody ratio. Insufficiency of reagents prohibited the testing of the supernatant with the antiserum.

Results

Table 2 presents the results of these tests. In the table (+) represents slight precipitate; (++++) represents heavy opaque precipitate; (–) represents no precipitate.

D. virilis antiserum. Referring to Table 2: before absorption, *D. virilis* antiserum precipitated with its homologous antigen to an antigen dilution of 1:640. After having been absorbed with *D. caribbea* antigen, the supernatant fluid reacted with the *D. virilis* antigen to an antigen dilution of 1:160. Consequently, *virilis* and *caribbea* hold fractions in common which produce precipitation at relatively high dilutions. By a removal of these fractions, the precipitating material held by *virilis*, to the exclusion of *caribbea*, is considerably less. In like manner, the *virilis* antiserum was absorbed with *D. mulleri* antigen. Upon testing the supernatant liquid with the *virilis* antigen, precipitation occurred only in antigen dilutions 1:20 and 1:30 (?). When *D. virilis* antiserum was absorbed with its homologous antigen, subsequent tests of the supernatant fluid with the same antigen were negative. Therefore, *D. caribbea* and *D. virilis* are seen to

TABLE 2

	Antigen dilutions											
	1: 20	1: 30	1: 40	1: 60	1: 80	1: 120	1: 160	1: 240	1: 320	1: 640	1: 1280	1: 2560
Virilis antiserum × *virilis* antigen												
Before absorption	++++	++++	++++	++++	+++	+++	++	++	+	+	–	–
After absorption with:												
caribbea antigen	++	++	++	+	+	+	+	–	–	–	–	–
mulleri antigen	+	±	–	–	–	–	–	–	–	–	–	–
virilis antigen	–	–	–	–	–	–	–	–	–	–	–	–
Caribbea antiserum × *caribbea* antigen												
Before absorption	++++	++++	++++	++++	++++	++++	+++	+++	+++	++	+	–
After absorption with:												
virilis antigen	++++	++++	++++	+++	+++	++	++	+	+	–	–	–
mulleri antigen	++++	++++	++++	+++	+++	++	++	+	+	–	–	–
melanogaster ag.	++++	+++	+	–	–	–	–	–	–	–	–	–
caribbea antigen	+	–	–	–	–	–	–	–	–	–	–	–

share a relatively small proportion of antigenic constituents, whereas *D. mulleri* and *D. virilis* share a large proportion of antigenic constituents. There are some fractions which *D. virilis* possesses which are not possessed by any one of the other species tested.

D. caribbea antiserum: This antiserum originally precipitated with its homologous antigen in dilution 1:1280. After having been absorbed with *D. virilis* antigen, the supernatant solution precipitated with the *D. caribbea* antigen in maximum antigen dilution of 1:320. Likewise, after having been absorbed with *D. mulleri* antigen, the supernatant fluid precipitated with the *D. caribbea* antigen in an antigen dilution of 1:320. Therefore, *caribbea* shares with *virilis* and *mulleri* approximately the same quantities of antigenic materials. These fractions are relatively small when compared with those shared by *caribbea* and *melanogaster;* for after the *caribbea* antiserum was absorbed with *melanogaster* antigen, the supernatant solution reacted to *caribbea* antigen in antigen dilutions no greater than 1:40. As in the case of *D. virilis, D. caribbea* possesses biochemical fractions not held in common with any one of the other species tested. The *D. caribbea* antigen did not completely absorb the *caribbea* antibodies in the antigen-antibody ratios indicated in Table 1. For this reason, there was a slight reaction in antigen dilution 1:20 when the supernatant liquid, produced by absorption with the homologous antigen and antiserum, was subsequently tested with that same antigen. This reaction is insignificant.

Discussion

An important observation should be made regarding the foregoing remarks: *D. virilis* and *D. mulleri* antigens give the same reactions to *D. caribbea* antiserum, in so far as this particular test is concerned. This does not imply that the same quality of materials is shared by *D. virilis* and *D. mulleri* with *D. caribbea.* Rather it implies that the quantity of materials shared by *virilis* and *caribbea* measured approximately the same as that shared by *mulleri* and *caribbea.* The same conditions would obtain regarding any other pair of antigens with reference to a given antibody. In this connection it may be well to mention the recent serological work of Irwin (1938) with dove and pigeon species. This investigator was able to prove that each species possessed biochemical

fractions not shared with any other species; and that a species possessed fractions which distinguished it from another species, but which in turn it could share with a third species. Presumably, the same situation exists among the *Drosophila* species.

Sturtevant (1921) placed *D. virilis* and *D. mulleri* in the same subgroup, whereas *D. caribbea* and *D. melanogaster* were placed together in another subgroup. He segregated these species into their respective categories on the basis of various morphological criteria, usually accepted as specific. In the present study *D. virilis* is shown to be closely related to *D. mulleri* and distantly related to *D. caribbea*. Likewise, *D. caribbea* is shown to be closely related to *D. melanogaster* and distantly related to *D. virilis* and *D. mulleri*. Obviously, the taxonomic and serologic methods have yielded much the same results. These data are sufficient to point out the particular advantages of this serological technic in elucidating the evolution of the *Drosophila* species.

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EFFECT OF TEMPERATURE UPON THE DEVELOPMENTAL RATE OF THE EMBRYO OF THE DIAMOND BACK TERRAPIN (*MALACLEMYS CENTRATA* LAT.)[1]

For several summers experiments have been conducted in an effort to discover whether or not the terrapin (*Malaclemys centrata* Lat.) egg follows the general rule of "cold-blooded" eggs in that the rate of development increases with increase of temperatures over a considerable range.

In the earlier studies a number of difficulties were encountered. Among these was the problem of securing freshly laid eggs in sufficient quantity. Although hundreds of eggs were available in the breeding pens, the terrapins were more or less sensitive to observers unless the oviposition had already been started, in which case the terrapin usually completed the act. When a terrapin was observed in the act of egg-laying, she was allowed to complete the process and was removed from the nest as soon as she began to "fill in." In numerous cases, however, the eggs were so thick in the pen that other nests were disturbed and eggs of an earlier laying became mixed with newly laid eggs. This problem was ultimately solved in the following manner. Parts of the laying pens were fenced off and opened 24 hours before the time for the collection of the eggs. The egg bed was closed off after the 24-hour laying period and thoroughly dug. This procedure was repeated at intervals during the laying season. By this means eggs were secured within 24 hours after laying. These were supplemented by eggs taken from uncovered nests. By this method a rather definite beginning point has been established. In some of the experiments a 48-hour period was used, since it gave many more eggs than two 24-hour periods, due apparently to the fact that terrapins lay better in a territory which they have been permitted to explore for a day or two.

Although taken from a single nest, the eggs show a considerable variation as to the degree of development. In a single oviduct of *Chrysemys cinerea* (Cunningham, 1922) stages from early cleav-

[1] The experiments were conducted at the U. S. Fisheries Laboratory at Beaufort, N. C., and the author expresses appreciation to the bureau for the use of its facilities and to Dr. H. F. Prytherch, director, and Mr. Charles Hastel, foreman, for their cooperation.

age to the late blastopore stage were found, and uterine eggs were taken in which the early flexure was beginning. These differences, however, seem to disappear during development and the eggs of a given clutch, as a general rule, appear to hatch at about the same time.

As has been pointed out in other papers, the problem of humidity, especially in artificially heated nests, was a serious one and even yet in sand nests has not been completely solved. At higher constant temperatures the eggs persist in drying out, even at relatively high humidity. This may account for the failure of eggs to develop at a constant high temperature.

Perhaps the most difficult problem was that of determining the end point. In earlier experiments the end point was taken as the time when the terrapin appeared at the surface. This proved to be a very unsatisfactory criterion, since the newly hatched terrapin remained under ground for quite variable periods of time after emergence from the egg. In our latest experiments the eggs were removed from the nest a few days before the calculated time for hatching and were reset on a bed of sand and covered with wet toweling which could be lifted and the eggs examined without disturbing them. The time of pipping the egg and of complete emergence from the shell could be fairly accurately determined. In our latest experiments the eggs were examined twice daily.

Experiments of last summer, reported elsewhere (Cunningham, Woodward and Pridgen, 1939), indicate that the whole of incubation may be carried out in battery jars without sand. Such procedure would lend itself to more careful temperature studies.

Last summer four distinct types of experiments, as related to temperature, were set up: (1) outdoor beds in which the normal environmental conditions existed; (2) indoor beds exposed to constant high temperature; (3) indoor beds with constant medium high temperature; (4) indoor beds at room temperature. The temperature of 1 and 4 were of course variable, that of 1 being regularly higher than 4. Since the results within each experiment, as well as between experiments, are quite concordant they are reported here.

In experiment 1 there were four beds involved. The recording thermometers showed but little difference in the *nest* temperatures. There were 190 eggs involved in this experiment. The time re-

quired between setting and hatching for the 4 groups is shown here.

Pen A.	61 days.	All eggs not hatched at this time were used for other purposes.
Pen B.	64–68 days.	
Pen C.	62–68 days.	
Pen D.	60–66 days.	

It is quite evident that in nature, under as nearly identical temperature conditions as possible, there is a considerable range of variation as to the time required for the newly laid egg to hatch.

In the second experiment a relatively high temperature of approximately 98° F. was maintained. The temperature never exceeded 105° F. nor fell below 95° F. The upper temperature is well below the maximum tolerance, since the beds in nature have been known to reach a temperature of 115° F. at the egg level without the death of any considerable number of the embryos. The lower level is considerably above the low level of the outdoor beds.

As in all our previous experiments on high temperatures the eggs failed to develop. Although having plenty of moisture, but not an excess, the eggs shriveled as if they were desiccated. From these experiments, which can not be considered conclusive, it would appear that terrapin eggs can not survive continued high temperatures, although they are lower than the maximum to which such eggs are exposed during normal incubation. If the embryos died without desiccation of the egg, one might attribute death to high rate of humidity, since terrapin eggs are rather sensitive to excess moisture.

In the third experiment the eggs were subjected to a fairly constant temperature of 85° F. There were 24 eggs in this lot, of which 23 hatched. The time required was from 61 to 68 days.

The fourth experiment consisted of two sets of eggs run at fluctuating room temperature ranging from 65° F. to about 92° F. The eggs were placed in battery jars without sand and in one experiment provided with distilled water, in the other with tap water (artesian well). The details of this experiment are described elsewhere (Cunningham, Woodward and Pridgen, 1939). There were 24 eggs receiving distilled water, all of which hatched. The range of hatching time was from 61 to 68 days. There were also 24 eggs receiving tap water, two of which were probably

infertile, the others all hatching. The hatching time was from 61 to 68 days.

In earlier experiments, which were not as well controlled, eggs which were never allowed to reach a temperature of 80° F. hatched in approximately the same time as those under normal conditions. Earlier experiments also indicated that development once started could be completely inhibited at ice box temperatures (approximately 55° F.) and embryos could be held in this condition for a period of at least two months. When returned to normal incubation temperatures such eggs develop in an apparently normal manner.

From the data presented it is evident that the rate of development of the diamond back terrapin does not fluctuate with environmental temperature, but that the rate is more or less constant through a wide range of temperatures. This is contrary to the generally accepted idea that in cold-blooded animals the rate of development is relative to the environmental temperature.

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